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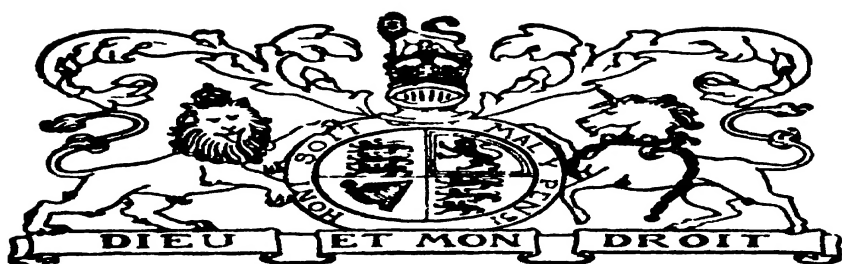
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**RECORDS OF THE MALARIA SURVEY  
OF INDIA.**



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# **RECORDS OF THE MALARIA SURVEY OF INDIA.**

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# MALARIOL

*Extract from "Statesman", May 13th, 1933.*

## MALARIA CONTROL

**OIL THAT KILLS LARVÆ IN FEW MINUTES.**

An important step towards wider control of malaria is announced by the Ross Institute in its annual report, in which is mentioned the introduction of an efficient standardized anti-malarial oil available throughout the Empire for oiling the surface of mosquito-breeding grounds. The new standard mixture kills off all larvæ within 15 minutes and some species within four minutes. The efficacy of the oil has been traced to certain chemical constituents, the proportions of which vary widely in normal oils and can be largely increased.

*The Ross Institute Report reads as follows :—*

"Dr. Ramsay replied that much research work had been done by the Ross Institute in conjunction with the Burmah-Shell group in London and Africa and by himself with the Assam Oil Co. at Digboi and the Burmah Oil Company in Burma. There was now a standard anti-malarial mixture available from these associated companies which kills off all larvæ within 15 minutes and some species in four minutes".

# MALARIOL

**is the standard mixture referred to above**

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# ERRATA.

Page 25, Table III, Col. 4, last line for 77 read 7·7.

„ 46, last line of footnote for 0·50 read 0·050.

BY

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Assistant Director, Malaria Survey of India.

[March 14, 1932].

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# INTRODUCTION.

THE inquiry into malarial conditions in Calcutta which forms the subject of this note was carried out during the period January 26th to February 25th,

1932. I wish to express my thanks to the following gentlemen for their assistance during the investigation :—

Major-General W. V. Coppinger, I.M.S., Surgeon-General with the Government of Bengal.

Dr. R. B. Khambata, Director of Public Health, Bengal.

Dr. S. N. Sur, Dr. Banamali Ghosh and Dr. H. Sarkar, Bengal Public Health Department.

Mr. F. C. Griffin, Chief Engineer, Bengal Public Health Department.

Mr. R. A. Senior White, Malariologist, Bengal-Nagpur Railway.

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## CHAPTER I.

### BRIEF DESCRIPTION OF CALCUTTA.

Calcutta is situated on the left bank of the Hooghly River, 86 miles from the sea. On the eastern aspect it is bounded by the Salt Lakes, which cover an area 10 miles in length and over three miles in width. The soil on which the city is built has been formed at a comparatively recent date by the alluvial deposits of the Gangetic Delta, and excavations made for tanks and foundations of buildings disclose alternate layers of sand and clay. The ground is only 18 to 21 feet above mean sea-level. The modern city was founded by Charnock in 1690.

Including the suburban areas which were added to the Municipality in 1924, the city covers an area of 31 square miles, with a population (including the Fort, Maidan, Port and Canals) of 1,196,668. The Municipal area is divided into four districts, which are subdivided into wards as follows :—

TABLE I.

District.	Ward.	Area in Acres.	Population.
I	1. Shampukur ..	430	66,643
	2. Kumartuly ..	199	38,294
	3. Burtolla ..	388	67,107
	4. Sukea Street ..	323	54,101
	5. Jorabagan ..	236	39,351
	6. Jorasanko ..	256	46,118
	30. Belgachia ..	548	20,077
	31. Satpukur ..	734	19,194
	32. Cossipore ..	765	27,213

*Malaria in Calcutta.*TABLE I—*concl'd.*

District.	Ward.	Area in Acres.	Population.
II	7. Burra Bazar ..	216	18,690
	8. Colootolah ..	226	48,998
	9. Moochipara ..	485	75,785
	10. Bow Bazar ..	136	21,075
	11. Puddopukur ..	153	35,323
	12. Waterloo Street ..	209	6,711
	28. Belliaghata ..	836	33,235
	29. Maniktala ..	1,338	42,399
III	13. Fenick Bazar ..	196	30,810
	14. Taltolla ..	184	38,427
	15. Collinga ..	194	14,052
	16. Park Street ..	164	5,438
	17. Baman Bustee ..	132	2,679
	18. Tangra ..	919	11,772
	19. Entally ..	526	44,224
	20. Beniapukur ..	429	40,587
	21. Ballyganj ..	810	30,765
IV	22. Bhowanipore ..	801	79,684
	23. Alipore ..	1,260	28,958
	24. Kidderpore and Ekbal- pore.	999	32,822
	25. Watganj and Hastings ..	837	32,552
	26. Garden Reach ..	3,441	55,872
	27. Tollyganj ..	1,344	40,730

**Population.**

The average density of population in the Municipal area is 58 persons per acre, but it varies greatly in different localities. Thus in Colootolah there are 217 persons per acre and in Taltolla 209. Nevertheless, overcrowding is by no means as serious a problem in Calcutta as it is in Bombay.

Immigration into Calcutta is very considerable, and it was stated at the Census of 1921 that only 335 persons per mille of the population were born in the city.

In 1921 nearly 71 per cent of the population consisted of Hindus, 24.5 per cent of Muhammadans and 3.25 per cent of Christians. The Muhammadans are most numerous in the eastern side of the city. The number of Europeans in Calcutta in 1921 was 13,192 and of Anglo-Indians 14,866.

As regards occupation the following information is taken from the Census of 1921 :—

TABLE II.

Occupation.	Number of workers and dependents.
Pasture and Agriculture .. .. .	78,000
Industry .. .. .	331,000
Transport .. .. .	120,000
Trade .. .. .	239,000
Public Force .. .. .	11,000
Public Administration .. .. .	40,000
Professions and Liberal Arts .. .. .	74,000
Persons living on their income .. .. .	15,000
Domestic service .. .. .	110,000
Insufficiently described .. .. .	276,000
Unproductive .. .. .	31,000

Of the 331,000 persons dependent on industry, about one-quarter were textile workers and their dependents, but nearly half of these belonged to Howrah. Of the commercial population, more than one-quarter were engaged in purveying food.

**Climate.**

The climate is hot and moist. The mean temperature averages 79°F., the mean maximum being 102° in May and the mean minimum 48° in January. The average temperature in the hot season is 85°, in the rains 83°, and in the cold season 72°. Humidity averages 78 per cent of saturation, ranging from 69 per cent in March to 89 per cent in August. The annual rainfall averages

60 inches, and the average number of rainy days in the year 118. Various climatic data for a number of years are given in Appendix A.

### **Vital Statistics.**

The death rate in Calcutta in the year 1929, the last year for which the annual report of the Health Officer of the Corporation is available, was 30·6 per thousand, calculated on the census population of 1921. The highest death rate was in Ward XIX (Entally), where the rate was 55. In this ward the death rate amongst females was 82 per thousand.

The principal causes of death were respiratory diseases (6·8), dysentery and diarrhoea (3·0), tuberculosis (2·6), cholera (2·4), 'other fevers' (1·7), and malaria (0·86).

The infantile mortality was 245 per thousand of registered births.

### **Water Supply.**

The drinking water supply is obtained from the Hooghly River at Palta, 17 miles north of Calcutta, where it is pumped up into settling tanks and filtered. The works have been greatly extended in recent years, and further extensions are still being made. The daily supply per head of the population is now 51 gallons. There is also an unfiltered water supply for flushing the drains and for watering the streets. Both filtered and unfiltered water are only supplied at high pressure at certain hours of the day, necessitating the use of an enormous number of cisterns and other receptacles for storing water.

### **Drainage.**

A scheme of underground drainage was commenced in 1859, and is completed throughout the city except in certain of the recently added areas. The water carriage system of conservancy has been increased very greatly in recent years, and is continually being extended. The drainage is carried eastwards into an intercepting sewer, and is thence raised to a high level sewer which carries it into the upper part of the Bidyadhari River close to the Salt Lakes.

### **Communications.**

Three great railways converge on Calcutta, the East Indian Railway connecting the city with Bombay, the United Provinces and the Punjab; the Bengal-Nagpur Railway with Orissa, Madras and the Central Provinces; and the Eastern Bengal Railway with North and East Bengal and Assam. The last mentioned railway connects Calcutta (Sealdah Station) with Diamond Harbour and Budge-Budge on the Hooghly River, and with Port Canning on the Matla River. Ulubaria, on the right bank of the Hooghly, is connected with the city (Howrah Station) by the Bengal-Nagpur Railway. The Kalighat-Falta Light Railway connects Calcutta (Majerhat Station) with Falta, on the

Hooghly; and the Barasat-Basirhat Light Railway connects Hasnabad, on the Ichamati River with the city (Shambazar Station).

In addition to the railway traffic, numerous country boats ply up and down the rivers, along the channels through the Sunderbans which connect Calcutta with Eastern Bengal and the valley of the Brahmaputra, and on the Midnapore and Orissa Coast Canals.

### **The Hooghly River.**

The Hooghly is the most westerly of the channels by which the waters of the Ganges enter the Bay of Bengal. During the rainy season the spill streams from the Ganges and the Chota Nagpur tributaries of the Bhagirathi pour down an enormous volume of water, which serves to scour out and maintain a deep channel. In the dry season, when there is no such influx, the river is largely fed by percolation, i.e., by the underground infiltration of water into the deep trough which the river has scooped out for itself. The Hooghly is a tidal river, and it is estimated that the tidal inflow during the four months of the dry season is more than double the total fresh-water discharge of the year. The greatest mean rise of the tide takes place in March, April and May, and is about 16 feet; there is a declining range during the rainy season to a mean of 10 feet, and a minimum during freshets of  $3\frac{1}{2}$  feet. The necessary depth of channel for shipping is maintained by means of dredgers.

The present channel of the Hooghly is very different from that which the Ganges formerly followed. The original channel was identical with Tolly's Nullah from Kidderpore to Garia, 8 miles south of Calcutta, from which point it ran to the sea in a south-easterly direction.

### **Canals.**

The more important canals in connection with Calcutta are as follows:—  
Tolly's Nullah connects the Hooghly with the Bidyadhari River.

The Kaorapukur Canal branches off from Tolly's Nullah a few miles south of Calcutta, and runs southward to Magrahat.

The Circular Canal extends from Chitpur Lock to the lock at Dhapa, and separates Maniktala from the rest of Calcutta. The section which forms the southern boundary of Maniktala is known as the Belliaghata Canal. A branch canal from Ultadanga, on the Circular Canal, to Dhapa is known as the New Cut Canal.

The Lake Channel is a tidal river running from Dhapa to Bamanghata, which has silted up considerably, and can only be maintained for the passage of boats by periodical silt clearance.

The Bhangar Canal extends from Bamanghata on the Bidyadhari River to Kulti Lock on the channel called the Kulti Gang.

The Kristopur Canal connects the New Cut Canal with the Bhangar Khal.



## CHAPTER II.

### THE HISTORY OF MALARIA IN CALCUTTA.

There is evidence that malaria has existed in Calcutta and its vicinity from very early times. Fry (1912) says 'The early records of the East India Company prove that Calcutta and Bengal were malarious from the very commencement of the British occupation'.

**1690.**—O'Malley (1914) quotes Wilson, Hamilton and Hunter as testifying to the ravages of fever at the close of the 17th Century. 'Within a decade after Charnock finally landed on the deserted river bank in 1690 it had become a busy mart with 1,200 English inhabitants, of whom 460 were buried between the months of August and January in one year. The miseries of the fever-stricken band throughout 1690 and 1691 are not to be told in words'.

**1756.**—In the autumn of 1756, just after Calcutta had been taken by Siraj-ud-daula, the British troops on board ship at Falta suffered very severely from fever. 'The exposure during the rainy season, coupled with bad food and other privations, brought on a malignant fever, which infected all the ships, and ultimately carried off a majority of the party, leaving the remainder in a wretchedly reduced and pitiable condition' (Orme, quoted by O'Malley, 1914).

**1880.**—Coming to more modern times, Iyengar (1931c) notes that in 1880 the Government of Bengal passed a resolution to the effect that the reclamation of the Saltwater Lakes is a project which 'the growing prevalence of fever in Calcutta' makes it desirable to see again brought forward.

**1899.**—In the report of the Health Officer of Calcutta for 1899 (Cook, 1899, quoted in *Lancet*, 1900) an account is given of anti-mosquito measures carried out 'to see whether any reduction could be made in the fever returns of Calcutta by giving some practical effect to what had hitherto been more or less theoretical considerations'. The services of certain Indians who had been trained by Major (now Sir Ronald) Ross, I.M.S., were obtained to hunt for anopheline larvæ, and the breeding places discovered (mostly tanks) were treated with kerosene oil. Dr. Cook concluded his report as follows:—'All things considered it does not appear probable that any appreciable effect can be made on the prevalence of malarial fevers in Calcutta with an expenditure of Rs. 30 a month, including pay of establishment, though that has been sufficient for a preliminary investigation into the prevalence of anopheles'.

The *Indian Medical Gazette* (Vol. XXXV, Oct. 1900, p. 400) commented somewhat caustically on the smallness of the amount sanctioned by the Corporation 'for the investigation of a disease which causes more mortality than all other diseases put together'.

**1900.**—In November 1900, the *Indian Medical Gazette* (Vol. XXXV, p. 443) comments on a sharp epidemic of malaria which occurred in the docks. 'The Port Commissioners and Messrs. Bird and Co. complained that the work of coaling at the docks was seriously interfered with by an epidemic of fever among the coolies. Dr. Cook and Capt. (afterwards Sir Leonard) Rogers visited the lines of these coolies and found that they were very well housed and that they were drinking filtered water, yet the fever which prevailed among them was of a distinctly malarial type. A ditch of stagnant water was found to run the whole length of the lines at a distance of only a few feet. In this ditch were found plenty of the anopheles larvæ. Messrs. Bird & Co. provided a barrel of tar, and the ditch was liberally treated with tar under Dr. Cook's own supervision on August 14th, and again on August 19th and 26th. However, we regret to say that the use of the tar had little or no effect either on the breeding of the anopheles or in preventing the cases of fever, for from a return of the number of cases of fever...it is clear that there was no falling off in the number of fever cases; for the average number of cases for 29 days before the first application of tar was 26 daily, and since that date the average number was 33, and after the second application of the tar 23 cases daily'.

It must be remembered that this was in the days when all that was known about the transmission of malaria was that it was carried by anopheline mosquitoes. The fact, first brought to light by Stephens and Christophers (1902), as the result of their investigations in India, that only certain species of anophelines carry malaria in nature, whilst others do not do so, was entirely unsuspected. The anopheline larvæ found on this occasion in the ditch of stagnant water were almost certainly *A. subpictus* ('rossii'), which as we now know is not a carrier in India; and the fact that the destruction of these larvæ had no effect on the incidence of malaria is therefore not surprising.

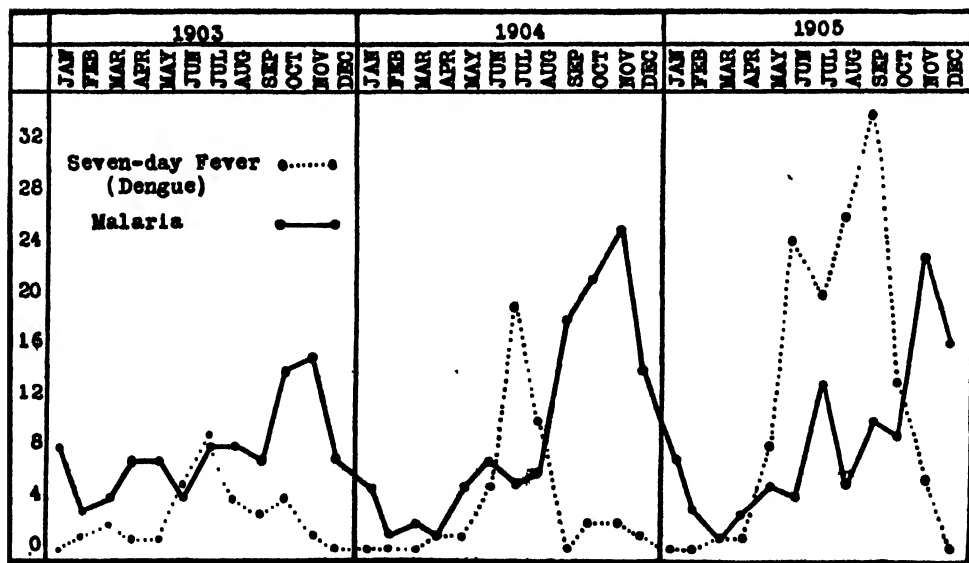
**1900.**—In February 1900, Rogers (1900) carried out an inquiry into the health of the tract of country just north of Calcutta and extending up along the east bank of the Hooghly as far as Naihati, with special reference to the prevalence of malaria. Two of the municipalities surveyed, Chitpore-Cossipore and Maniktala, are now within the municipal limits of Calcutta. The spleen rates found there were 11·2 and 13·2 respectively, being very considerably lower than in areas further to the north and east. Rogers notes that these two municipalities were the most water-logged in the area surveyed, the ground water levels being from 4 to 5 feet below the surface in the dry weather, and from one to two feet during the rains.

**1901.**—In 1901 Drs. J. W. W. Stephens and S. R. (now Sir Rickard) Christophers visited Calcutta as members of the Malaria Commission of the Royal Society. Coming as they did directly from a tour in West Africa, where spleen rates were everywhere very high, they were at once impressed by the absence of splenomegaly among the children of Calcutta, in spite of the

prevalence of anophelines (chiefly *A. subpictus*) in large numbers (Stephens and Christophers, 1902).

The number of children examined by the Commissioners was however very small, 191 in all. Of these 57 were examined in Phoolbagan Road, 77 in Hastings, 7 in Fort William and 50 in Kidderpore. None of these were found to have enlarged spleens, or to have malaria parasites in their blood. Most of the observations (141) were made in June, July and August, and the remainder in September. Dissections of 324 anopheline mosquitoes were made with negative results.

The Commissioners also noted that though during the period of their visit many cases of enlarged spleen were admitted into the different civil hospitals of Calcutta and diagnosed as malaria, yet parasites were only found with the utmost rarity. 'The only hospitals in which parasites were readily found by us were the military hospitals'. The explanation of this is no doubt that a large proportion of the patients with enlarged spleen in the civil hospitals were cases of kala-azar, coming from various parts of Bengal.



Graph illustrating the seasonal prevalence of dengue and malaria in Calcutta, 1903-1905 (after Rogers).

The figures given by James (1902), often quoted as separate observations, refer to the results obtained by Stephens and Christophers noted above. James notes that in June the only places where anopheline larvæ were found in one part of Calcutta (Hastings) were cisterns on the roofs of the houses.

**1903-5.**—Rogers (1906) published an important paper dealing with malaria fever and its differentiation from 'seven-day fever' (dengue), based on observations made in the years 1903, 1904 and 1905 (*see* Graph, p. 10). The following extract is taken from this :—

'With regard to the cases coming from Calcutta itself, some may doubtless have contracted the disease elsewhere and suffered from a relapse while subsequently living in Calcutta, but the histories of a considerable number of them point very definitely to their having first got the disease while actually residing in the town. Among such cases are a number of sailors who had not previously suffered from malaria, but who contracted the disease within a few weeks of coming to this port in the malarial season, which will not be surprising to anyone who knows the state of Kidderpore Docks and its surroundings. Again, several nurses of the General Hospital contracted the disease for the first time while living in quarters within the hospital compound, and other similar undoubted cases of first infection by malaria in Calcutta might be given. Again, the disease very often attacks persons from parts of Australia which are free from malaria, but who are residing in Garden Reach in connection with the trade of importing horses. Yet the examination of blood films from a large number of healthy children in various parts of Calcutta, including both Kidderpore and Garden Reach, made by Captain (now Major-General) Megaw, I.M.S., and myself during the last two years (details of which will be published elsewhere\*) have confirmed the fact pointed out by the Malaria Commission that the so-called "endemic index of malaria" of Calcutta is *nil*, and that too in spite of our examinations having been made at the height of the malaria season in the month of November, instead of the hot weather or early rains as in the previous observations referred to. It is clear then that an "endemic index of malaria" of *nil* is not evidence that malaria is absent from a place, although doubtless it may mean that the place is not an extremely malarious one, although Garden Reach appears to be an exception even to this modified rule; for the disease has been so rife among the police there as to call for special investigation last year, while in the present year (1905) only three out of 18 police stationed there have escaped the disease. Caution must then evidently be exercised in accepting a negative "endemic index" as a certain indication of the absence of malaria from any given place'.

The places from which the malarial cases came were :—

Calcutta (within municipal limits)	..	..	70
Suburbs of Calcutta—(Garden Reach)	..	..	15
(Tollyganj)	..	..	5
Surrounding districts	..	..	23
Railway employees	..	..	24
Miscellaneous	..	..	17

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\*I have been unable to trace these figures in any publication.—G. C.

Rogers concluded that malarial fevers in Europeans frequently originate in Calcutta in spite of the so-called 'endemic index of malaria' being *nil*, their main prevalence being from October to December, during the drying up to the end of the rains; and that seven-day fever (dengue) occurs every year in Calcutta in the early rainy season, and has a different seasonal incidence from malaria.

**1906.**—Another very interesting paper was published by Megaw (1907), recording the results of a year's blood examinations made among out-patients at the Medical College Hospital. He states 'the most careful enquiries were directed to ascertaining the probable locality in which the infection had been contracted; and, after eliminating all doubtful cases, there remain numerous instances in which the infection was undoubtedly acquired in Calcutta, many of the patients never having been away from the neighbourhood of Bow Bazar all their lives'. He gives the following table showing the probable origin of infections :—

		Calcutta and Suburbs.	Mofussil.
Malignant tertian	..	.. 97	93
Benign tertian	..	.. 61	52
Quartan	..	.. 7	30
		<hr/>	<hr/>
	Total	.. 165	175
		<hr/>	<hr/>

**1911.**—In November 1911 the second meeting of the General Malaria Committee was held at Bombay. At this meeting Dr. K. C. (afterwards Sir Koilas) Bose made the following remarks (Bose, 1911):—

'Calcutta has at present in the Bara Bazar a district that is very bad with malaria cases. There is not a single house where ten cases could not be seen, and the cases are all of the tertian type. There are a few cases of malignant tertian.... In trying to find out the breeding-places of the carriers in this neighbourhood, the speaker was surprised to find *anopheles* larvæ in coconut shells in the houses'.

**1912-16.**—From August 1912 to January 1913 Major McGilchrist made a *Stegomyia* survey of the port of Calcutta. James (1913), in a paper on the practicability of *Stegomyia* reduction in Indian seaports, with a view to the protection of India from yellow fever, suggested the provision of a constant high pressure water supply as the first step that should be taken to reduce these mosquitoes. In 1914-15 Assistant Surgeon Nandi carried out a *Stegomyia* survey in two selected areas in Calcutta, and a summary of his findings was given in the report of the Health Officer, Calcutta, for the year 1915. In the same year Christophers (1915) put forward suggestions for the creation of an

organization for reducing the numbers of *Stegomyia* mosquitoes in Calcutta. His proposal that a complete scientific survey of mosquitoes extending over five years should be undertaken was endorsed by a special conference convened to frame a scheme for securing better sanitary control over the port of Calcutta and its environs. The conference also suggested that the survey should not be confined to *Stegomyia* only, but should be extended to mosquitoes of all classes which convey disease. In an appendix to the report of the conference Dr. C. A. Bentley laid down detailed recommendations for the prevention of mosquito-breeding in Calcutta (Bentley *et al.*, 1918).

**1911-1930.**—A study of the annual reports of the Health Officer, Calcutta, for the past 20 years shows that malaria has been considered to occur chiefly in the southern and south-eastern wards of the city. From 1916 to 1923, however, certain of the central wards, notably Ward XI, appeared in the list of the most malarious wards. In the reports for certain years there are records of spleen examinations made in various areas. In 1915, the spleen rate in Ward XX was 6·8 per cent (1,158 observations). In 1916 the rate in Ward XIX was 1·26 per cent (2,454 observations), in Ward XXI 2 per cent (1,016 observations), and in Ward XXIV 2·5 per cent (738 observations). In 1918 the rate in Districts III and IV was 3·2 per cent (407 observations) and in 1919 the same figure was recorded (1,040 observations). In 1920 the high figure of 24·7 per cent (1,621 observations) is given for these two districts. This latter figure is by far the highest ever recorded for any part of Calcutta. There is no other evidence that malaria was particularly rife in Calcutta or in the rest of Bengal in that year, and there is considerable doubt as to the accuracy of this record. Unfortunately, no records of spleen examinations are given in the annual reports for subsequent years.

**1917-1919.**—Iyengar (1920) published an account of investigations in Calcutta, from which the following is an extract :—

‘During recent years, medical men in Calcutta have observed that malaria is to some extent prevalent in the city, and that people who have never left Calcutta have contracted a virulent type of malaria. Many medical men believe that malaria has been increasing during the last few years, and malarial fever is certainly very common in the suburbs at present, though the spleen index is low’. He noted that the splenic index in parts of Tollyganj was from 4 to 10 per cent, averaging 5 per cent, in Kidderpore 2 per cent, in Sibpur 2 per cent, and in Entally *nil*. He showed that *A. stephensi*, the notorious malaria carrier of Bombay, Madras and many other cities in India, was very prevalent in Calcutta, and was breeding in large numbers throughout the city. ‘The principal breeding-places are the cisterns for storing filtered and unfiltered water on the house terraces. In every house in Calcutta there is at least one cistern for storing unfiltered water for use in flushing the lavatory; and in others there are besides several cisterns for storing filtered water. Garden

tubs, old unused cisterns and shallow pits on terraces form abundant breeding-places'. In certain parts of the city more than 60 per cent of the cisterns examined contained larvæ of *A. stephensi*. 'So long as the present conditions are allowed to exist, Calcutta runs the risk, sooner or later, of being over-run by malaria. The city is developing rapidly, and this development is leading on the one hand to the increase of the potential breeding-places for *A. stephensi* in all directions, and on the other to the influx of workers from many places, a proportion of whom are almost certain to be already infected with malaria. If this sort of thing continues we may suddenly find, as the result of an epidemic of malaria, as they did in Bombay a few years ago, that Calcutta has become malarious almost before anyone is aware of the fact. Two points require to be emphasized; firstly, a more complete mosquito survey of Calcutta is essential and, secondly, necessary measures for the control of *A. stephensi* breeding in Calcutta should be carried out, supported by adequate legal powers. The best anti-*stephensi* measure would be to introduce a continuous water supply. This would get rid of the cisterns, and thus solve the *Stegomyia* question as well'.

**1923.**—De (1923) states 'there occur plenty of cases of malarial fevers in Calcutta every year. We concede that a certain percentage of the total is imported, but it is difficult to escape the conclusion that a very large percentage is certainly due to local infection. The very large numbers of blood smears collected mostly from District IV and examined by me revealed sexually mature parasites in nearly 50 per cent of the cases. Fresh cases of malaria were cropping up continually until the case incidence reached the *fastigium* after the rains, the old cases having served as potential foci in the spread of infection'.

**1928-29.**—Basu (1930) records the result of a mosquito survey made in an area one square mile in extent in Central Calcutta, bounded by Machua Bazar Street and Cotton Street on the north, Bow Bazar Street, Lal Bazar Street and Dalhousie Square North on the south, Amherst Street on the east, and Charnock Place and Clive Street on the west. Larvæ of *A. stephensi* were found in 1,131 breeding-places out of 4,119 examined, the 'chief being masonry cisterns (29 per cent infected), galvanized iron cisterns (30 per cent), wooden barrels (24 per cent), earthen *handis* (27 per cent), earthen tubs (23 per cent), earthen jars (29 per cent), kerosene tins (28 per cent), tin mugs (22 per cent), iron pans (30 per cent), iron tubs (17 per cent), wooden boxes (35 per cent), empty pitch barrels (38 per cent). With regard to cisterns, Basu states:—'These are made of galvanized iron sheets of standard thickness, and are mostly used as reservoirs for storage of unfiltered water to flush latrines, etc. Every house has at least one on its roof; their capacity is proportional to the number of latrines in the house, the Corporation rules demanding a capacity of 60 gallons per latrine.

In the area under consideration most of the buildings are old type Indian quarters; the kitchens are on the ground floor in the same block as the dwelling rooms; and middle-class Indians, as a rule, instead of using gas or electric stoves, burn coke and wood for cooking purposes. As a result of the repeated warnings of the Smoke Nuisance Committee, the inhabitants of these buildings are gradually shifting their kitchens to the roofs of their houses. This results in an increased demand for filtered water on the roof, but as the pressure of the filtered water supply is only a few feet in the city, people collect filtered water in a masonry cistern in the ground floor and thence pump it into a cistern on the roof. On enquiry the dealers in plumbing material and goods informed me that the sale of pumps and cisterns is rapidly increasing in the city. Thus, the introduction of roof kitchens may lead to smoke abatement, but unless there is a sufficiently high and continuous pressure of filtered water, this will add to the danger of *A. stephensi* breeding in Calcutta.... It is abundantly clear that the reservoirs of filtered and unfiltered water in the centre of Calcutta city are the main source of the *A. stephensi* breeding in the city. These constitute such danger with regard to malaria transmission as may exist; they present a very much more serious danger with regard to the transmission of dengue by *Aedes aegypti* (and even possibly of yellow fever, if introduced) '.

**1930-31.**—In the autumn of 1930 there were severe outbreaks of malaria at Budge-Budge and Chengail, situated on the left and right banks of the Hooghly respectively, within 12 miles of Calcutta. The outbreaks were investigated by Mr. Iyengar and Dr. Sur, of the Bengal Health Department, and *A. ludlowi* was incriminated as the transmitter of malaria in these areas, an infection rate of 24 per cent being found in this species at Budge-Budge, where the epidemic was particularly severe (Iyengar, 1931a; 1931b). In a foreword to Mr. Iyengar's report, Dr. C. A. Bentley, then Director of Public Health, Bengal, wrote as follows:—'I cannot too strongly emphasize the importance of checking the spread of this dangerous species of *Anopheles*, as the Port and the City of Calcutta and the mills with a large amount of capital and labour are situated in an area which is potential for the breeding of the species. They will be in danger if once *Anopheles ludlowii* settles in the salt lake area, and in the slightly saline tanks and ponds along the river course.... I think that if the problem is not taken up in right earnest in its present stage, it may reach an enormity with which it would be practically impossible to deal with '.

In March 1931 a conference was held by the Hon'ble Minister in charge of Public Health, Government of Bengal, to discuss the danger of the spread of *A. ludlowii* and the preventive measures necessary. After this conference the Director of Public Health secured sanction for the employment of a suitable staff for the survey of the area concerned at a cost of Rs. 7,500, and the Local



Government sanctioned a sum of Rs. 13,000 for control measures. A sub-committee was formed for watching the position and co-ordinating anti-malarial measures. This sub-committee held its first meeting on July 20th, 1931, and subsequent meetings in September and November 1931 and in January 1932. At these meetings the reports of the survey and control units of the Department of Public Health and of the officers in charge of anti-malaria measures of the Bengal-Nagpur and Eastern Bengal Railways were considered, and the measures found necessary for the investigation and control of the situation were decided on.

*A. ludlowii*, which is a brackish water breeder, had not previously been recorded so near Calcutta (except on one occasion, which will be referred to later), though it had been found breeding at Taki in the Basirhat Subdivision and at Port Canning and various localities in the Sunderbans. Larvæ of this species were subsequently found at Falta, 12 miles below Budge-Budge on the Hooghly, where a severe outbreak of malaria occurred in the autumn of 1931, at Ulubaria, 5 miles below Budge-Budge, at Manshapukur and Bansra on the river Bidyadhari, 20 miles from Calcutta, and at Hasnabad and villages in its vicinity on the river Ichamati, 40 miles from Calcutta. An outbreak of malaria occurred in the same year at Port Canning, and *A. ludlowii* was found breeding there in large numbers. Larvæ of this species were also found in other villages in the neighbourhood.

Large numbers of adult specimens of *A. ludlowii*, of which 8 per cent were found to be infected with malaria parasites, were captured in train coming from Falta to Majerhat, which is the terminus of the Kalighat-Falta Railway, and which is situated at the edge of the extensive swamps lying immediately to the south of Kidderpore Docks. The danger of this species breeding in these swamps and giving rise to an epidemic of malaria in the docks was obvious, and in the autumn of 1931 the Garden Reach Anti-Malaria Association started control work there as a safety measure by paris green dusting from an aeroplane. Larvæ of *A. ludlowii* were not found breeding in the swamps, but adult specimens were caught in the catching stations in Kidderpore Docks and Dumayne Avenue early in November, and on November 12th a male insect was captured at Bracebridge. It is possible that these specimens may have originated from breeding-places in the vicinity, although none were detected.

Adult specimens of *A. ludlowii* have also been caught at Howrah, in trains from Ulubaria, at Shambazar in trains of the Barasat-Basirhat Light Railway, and at Sealdah Station in trains from Budge-Budge and Port Canning. This species has also been taken on various occasions in country boats coming up the Kristapur Canal and elsewhere. Adult specimens were caught in considerable numbers at Shamnagar, 19 miles north of Calcutta on the left bank of the Hooghly in 1930, suggesting that they were breeding in the vicinity, but

larvæ were not discovered. Neither adults nor larvæ have been captured there since that date (Map III).

In November 1931 a deputation from the Bengal Chamber of Commerce waited on the Governor of Bengal and presented a note drawing attention to the outbreaks of malaria at Budge-Budge and Chengail and to the fact that malaria was unusually severe in Calcutta itself, and pointing out the danger of a serious epidemic of the disease occurring in the city as the result of the presence of *A. ludlowii* on its outskirts and of *A. stephensi* throughout the city. The note contained the outline of a scheme for the investigation and control of malaria in Calcutta and for a radius of five miles beyond its boundaries.

In December 1931 the situation as regards malaria in Calcutta and its vicinity was discussed by the Malaria Sub-Committee of the Conference of Medical Research workers, and a resolution was passed recommending an inquiry to be undertaken by an officer of the Malaria Survey of India.

At the request of the Government of Bengal, the present writer was detailed to undertake an investigation of one month's duration, and the inquiry was commenced on January 26th, 1932.

## CHAPTER III.

### ANTI-MALARIA MEASURES CARRIED OUT IN CALCUTTA.

#### Work carried out by the Municipality.

The anti-larval measures carried out in the years 1899 and 1900 by Dr. H. N. Cook, Health Officer of Calcutta, have already been referred to. They consisted chiefly of applying kerosene oil to tanks in which anopheline larvæ were found.

In the annual report of the Health Officer of Calcutta for the year 1929 (Majumdar, 1931) we find the following information under the head of 'Anti-Malarial Campaign—Mosquito Brigade'.

'The question of starting a comprehensive anti-mosquito campaign generally and anti-malarial campaign specially has been engaging the attention of the Corporation since 1909. Originally a small temporary staff of mosquito brigades was appointed, which worked about 6 months of the year from October to March. In 1916 a proposal to re-organize and strengthen the mosquito brigades and to undertake systematic anti-malarial measures in selected areas was considered by the Corporation, and the following temporary staff for anti-malarial measures was sanctioned :—

2 Inspectors.  
24 Sub-Inspectors.  
72 Coolies.

'This staff was divided into 2 brigades and posted in certain selected areas of Districts III and IV, which were most severely affected with malaria. In the meantime Dr. Bentley, the Director of Public Health, Bengal, sent in a comprehensive scheme on anti-mosquito measures in Calcutta, and considerable attention was paid to this proposal. In May 1917 the Corporation considered the scheme, and placed the temporary staff on a permanent footing. Nothing was done to give effect to Dr. Bentley's larger and more comprehensive scheme.

'The brigades thus sanctioned continued to work, but in January 1923 the Establishment Committee recommended the abolition of the staff from April 1st, 1923. The Corporation in June 1923 accepted the recommendations of the Establishment Committee, and the mosquito brigade staff was for the time being abolished. The question of starting anti-malarial operations was again brought up before the Corporation in September 1927 when the appointments of

- (1) One qualified medical man with training in Entomology and Tropical Medicine,

- (2) One qualified Medical Inspector,
- (3) Twelve Sub-Inspectors, and
- (4) Seventy-two Coolies

were sanctioned.

'The staff sanctioned for the town proper was appointed partly in February and partly in March 1928, and has since then been working as a temporary measure from year to year. The staff is divided into 2 brigades, one working in District III and the other in District IV. In addition to this a brigade consisting of 2 Sub-Inspectors and 16 coolies was sanctioned for Maniktala, and has been working since July 1929'.

This staff was subsequently increased, and in November 1931 it was composed as follows :—

*District III.*

- 1 Inspector on Rs. 80 plus Rs. 10 bicycle allowance.
- 6 Sub-Inspectors at Rs. 40 each.
- 36 Coolies at Rs. 14 each.

*District IV.*

- 1 Inspector at Rs. 90 plus Rs. 10 bicycle allowance.
- 6 Sub-Inspectors at Rs. 40 each.
- 36 Coolies at Rs. 14 each.

*Districts I and II.*

- 2 Sub-Inspectors at Rs. 40 each.
- 12 Coolies at Rs. 14 each.

*Maniktala.*

- 2 Sub-Inspectors at Rs. 40 each.
- 16 Coolies at Rs. 14 each.

For Districts I and II and Maniktala, 1 Mosquito Inspector at Rs. 90 plus Rs. 10 bicycle allowance.

In 1930 a scheme for mosquito control in Calcutta was drafted by Dr. K. S. Ray, Chairman of the Public Health Committee, to deal with 32 wards, including the added areas in Calcutta.

The scheme was discussed at a Conference held on December 11th, 1930, and was generally approved. It was placed before the Public Health Committee on February 12th, 1931, and it was resolved that the scheme should be approved with certain modifications. The Public Health Committee at their meetings held on July 16th and 18th, 1931, considered the matter, and passed the following resolutions :—

(a) That Dr. K. S. Ray's scheme for mosquito control be approved and adopted with the following modifications :—

That the establishment be as follows :—

	Mean pay per month.
	Rs.
Each unit should consist of 1 Sub-Inspector on Rs. 40-50 ..	45
2 Sircars each on Rs. 20-25 .. ..	45
6 Coolies each on Rs. 14 .. ..	84
	<hr/>
	174
	<hr/>
For 32 wards the expenditure would be $32 \times 174$ ..	5,568
10 additional units for those wards which are bigger and have got a larger number of breeding-places .. ..	1,740
4 Inspectors on Rs. 80, plus Rs. 10 cycle allowance each ..	360
1 Mosquito Controlling Officer on Rs. 400-20-600 plus Rs. 100 motor car allowance .. ..	500
4 Surveyors for the first two years, after which only one will be retained on Rs. 60 per month .. ..	240
1 Laboratory Assistant on Rs. 50 per month ..	50
2 Clerks on Rs. 40-125 each .. ..	165
1 Entomologist for the Laboratory on Rs. 150-250 ..	200
1 Peon for Mosquito Controlling Officer on Rs. 14-20 ..	17
	<hr/>
Total monthly establishment ..	8,840
	<hr/>
Other recurring expenses :—	
Crude oil .. ..	800
Cresol .. ..	100
Contingencies including telephone .. ..	200
	<hr/>
Total ..	1,100
	<hr/>
Total monthly charges ..	9,940
	<hr/>
or say, ..	10,000
	<hr/>
Total annual recurring charges	1,20,000
	<hr/>
Non-recurring charges, furniture and equipment ..	1,000
	<hr/>

(b) That an advisory committee consisting of the following members :—

1. Director of Public Health, Bengal,
2. Director of the All-India Hygiene and Public Health Institute,
3. Malariologist of the Eastern Bengal Railway,
4. Malariologist of the Bengal-Nagpur Railway,

5. Health Officer of the Corporation,
6. Seniormost District Health Officer of the Corporation,
- 7-9. Three representatives of the Public Health Standing Committee (excluding the Chairman and Dr. K. S. Ray),
10. The Chairman of the Public Health Standing Committee.
11. Dr. K. S. Ray,
12. Chairman of the Calcutta Improvement Trust,
13. A representative of the Indian Medical Association,
14. A representative of the Calcutta Medical Club,
15. A representative of the Corporation,

be constituted to advise in regard to the general policy to be followed in regard to mosquito control.

The Mosquito Controlling Officer should be the Secretary of the Committee. The proceedings of the minutes of the committee should be forwarded to the Health Committee.

(c) That the qualifications for the different posts suggested in the scheme be prescribed as follows :—

(1) The Mosquito Sub-Inspectors should be persons who have passed Sanitary Inspector's Examination from the Bengal Public Health Department or from the Royal Sanitary Institute of Bombay, or who are passed students from any recognized Medical Institution.

(2) The existing Mosquito Sub-Inspectors should pass an examination regarding identification of mosquitoes and methods of control, etc.

(3) The Mosquito Inspectors should be L.M.F.'s or L.M.P.'s who have also passed the D.T.M. examination of the School of Tropical Medicine, Calcutta, or M.B.'s or L.M.S.'s.

(4) The existing Mosquito Inspectors should also pass an examination in Hygiene and Entomology, before they are taken in permanently.

(5) Regarding the Entomologist, he should be an L.M.S. or M.B. or M.Sc. in Zoology with special training in Entomology in each case.

(6) Regarding the Mosquito Controlling Officer, he must be an L.M.S. or M.B. with D.P.H. or D.T.M., with previous experience and administrative capacity.

The Corporation may however relax any of these rules in regard to any candidate found suitable for any of the above posts.

(d) That as regards propaganda work, the work be done by the Publicity Section of the Health Department, for which a special allotment has been made in the current year's Budget.

*N.B.*—In Dr. Ray's original scheme provision was made for 4 lorries with drivers. The total yearly recurring charges were to be Rs. 1,20,000, and the non-recurring were to be Rs. 12,500, including Rs. 10,000 for the 4 lorries.

**Comment on the Anti-Mosquito Scheme of the Calcutta Corporation.**

(i) It appears that the object of the organization is not to deal especially with mosquitoes which convey disease, but to attempt to control *all* mosquitoes in Calcutta. To accomplish this successfully would need a staff many times as large as that at present envisaged, and an annual recurring expenditure running into many lakhs of rupees.

As is shown later in this note, the only malaria-carrying mosquito which is to be feared in Calcutta is *A. stephensi*, which breeds especially in cisterns and other receptacles for storing water, garden tubs, fountain basins, disused tins, pots and barrels in which water has collected, and other similar situations. There are also the favourite breeding-places of *Aedes aegypti* (*Stegomyia fasciata*), which is the carrier of dengue and of yellow fever, and the bites of which are more irritating than those of any other mosquitoes in Calcutta.

It is therefore strongly urged that the efforts of the anti-mosquito organization be directed in the first place against the breeding-places of these two species of mosquito. In this way the transmission of malaria and of dengue will be reduced, the danger of a possible outbreak of yellow fever minimized, and the 'mosquito nuisance' largely decreased. If the efforts of the organization at its present budgeted strength are to be directed against all mosquitoes indiscriminately, there is a great probability that its efforts will be largely wasted, and that it will fall into disrepute.

(ii) *Training of staff.*—The control of mosquito breeding and of mosquito-borne disease is a very specialized branch of medical science, and it is very necessary that the staff of the organization shall be efficiently trained. It is suggested that the Controlling Officer shall be sent to attend the annual Malaria Course held by the Malaria Survey of India at the Field Experimental Station, Karnal, and that the Director of Public Health, Bengal, be asked to allow this officer and the inspectors under him to accompany his anti-malaria staff and to learn their methods of field work\*. The success of the scheme depends almost entirely on the practical knowledge, keenness and energy of the controlling staff, and no amount of office work and administrative ability can compensate for lack of knowledge of the practical details of survey work and control measures.

(iii) It is also recommended that the organization shall undertake an annual spleen census of municipal school children, throughout the city, particular care being taken to exclude those who have recently arrived in Calcutta, or who have visited places outside Calcutta during the previous twelve months.

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\* Since the malaria problem in Calcutta is in many respects similar to that in Bombay, it would be of great benefit if the Controlling Officer could visit Bombay and study the malaria organization in that city. The Health Officer, Bombay Municipality, has expressed his willingness to offer every facility for such a visit.

These figures should be recorded in the annual report of the Health Officer by wards, or better still by individual schools. In this way a very valuable guide will be obtained as to the increase or decrease of malarial incidence in the various parts of the city. Further evidence of this could be obtained by the examination of blood films from, say, 100 children in each ward carried out at the same time each year, as has been done in the course of the present investigation. The parents of all children found to have evidence of malarial infection should be notified, and advised to have their children medically treated.

Another very valuable source of information would be provided if the superintendents of the various hospitals and dispensaries in the city were asked to keep a separate record of those cases of malaria which are obviously locally acquired, as distinct from those which have resulted from infection acquired elsewhere.

The police, military authorities and railway authorities should be asked to furnish similar figures, and all the data thus received should be included in the annual report of the Health Officer of the Calcutta Corporation.

#### **Work carried out by other bodies.**

In 1929 and 1930 anti-mosquito work was carried out by the Bengal-Nagpur Railway authorities at their headquarters colony at Garden Reach. In 1931 the Garden Reach Anti-Malaria Association was formed, consisting of the following members :—

The Calcutta Port Commission,  
Messrs. Mackinnon, Mackenzie & Co., on behalf of the B. I. S. N. Co.  
and Macneill & Co.,  
The Bengal-Nagpur Railway,  
The Calcutta Electric Supply Corporation Ltd.,  
The North-West Soap Co., Ltd.,  
Messrs. Bird & Co.

The primary aim of the Association has been an anti-malaria campaign, but the measures adopted have been directed against all mosquitoes, with the object of minimizing the incidence of dengue as well as of malaria, and of reducing the general mosquito nuisance. The area controlled by the Association amounts to approximately 613 acres, extending from Kidderpore No. 1 Dock on the east to the Southern Power Station of the Calcutta Electric Supply Corporation on the west, beyond King George's Dock; the Hooghly and Garden Reach Circular Road forming the remaining boundaries. On the south the fringe of the Matiabruz swamps beyond Garden Reach Circular Road is also dealt with. In addition to this area two subsidiary controls are operated; (i) on the Ferry Steamers and Ferry Pontoons on the Howrah shore, and



(ii) in Kidderpore Dock No. 2 and on B. I. S. N. ships in the Docks and at River Moorings. The latter work is carried out by a Sub-Assistant Inspector and a small staff entertained by Messrs. Mackinnon, Mackenzie & Co., supervised from the office of the Association. Within the Association's area there are located 558 breeding-places, varying from large swampy areas to cesspools, which are regularly treated. Regular weekly catching of adult mosquitoes is carried out in 11 catching stations in the area (see Chart, p. 29).

The expenses in connection with the work are met by subscriptions from the various members of the Association, which amount to Rs. 625 per month.

The staff employed consists of 1 Inspector, 2 mates and 8 coolies. Laboratory and clerical work is undertaken by various members of the staff of the Malariologist of the Bengal-Nagpur Railway, who receive small monthly allowances from the funds of the Association.

In the autumn of 1931, the Majerhat swamps were treated with paris green partly by hand and partly from an aeroplane, emergency funds for this purpose being supplied by means of a special grant from Messrs. Mackinnon, Mackenzie & Co. and the Port Commission.

The Eastern Bengal Railway authorities carried out work at Budge-Budge in 1931 in co-operation with the Budge-Budge Municipality and the Bengal Public Health Department. The Jute Mill authorities at Budge-Budge co-operated by draining certain low-lying areas and borrow-pits and pumping the water into the Hooghly. Anti-larval work has also been undertaken by the Eastern Bengal Railway at Majerhat and Port Canning, and catching of adult mosquitoes has been carried out at Majerhat and Sealdah Stations and in other localities. The staff employed by the Eastern Bengal Railway for this purpose consists of 1 European Inspector, 1 Jemadar, 4 oiling coolies, a sanitary gang of 6 men and 1 mate for clearing jungle, and a special gang of 6 men for clearing borrow-pits and tanks from algæ and other vegetation. In addition, one Dispenser has been employed for distributing quinine and for taking blood films from railway employees and their families. The whole staff works under the supervision of the District Medical Officer, Calcutta. The total expenditure on anti-malaria work by the Eastern Bengal Railway in Calcutta, Port Canning and Budge-Budge from April to December 1931 (excluding the pay of the Dispenser) was Rs. 3,575.

As has been mentioned above, a special grant of about Rs. 14,000 was made by the Government of Bengal for anti-malarial measures. These have been carried out at Budge-Budge, Falta and Chengail by the staff of the Bengal Health Department.

## CHAPTER IV.

### THE PRESENT AMOUNT AND DISTRIBUTION OF MALARIA IN CALCUTTA.

The degree in which malaria is present in any community is best measured by the examination of children living in the locality for enlargement of the spleen and for the presence of malaria parasites in the blood. The procedure followed in the present investigation was to examine as many children as possible for splenic enlargement, and to take blood films from 100 children in each ward of the city. The subjects examined were chiefly children attending the Municipal Schools, aged from 6 to 10 years, this being the only method of making a significant number of observations in the limited time available. Altogether 97 schools were visited. In addition, children between the ages of two and ten years were examined from among the employees of the Bengal-Nagpur Railway at Kidderpore Docks and Shalimar (Howrah), and also from among the employees of the Hooghly Mill at Kidderpore Docks. Altogether 8,945 children were examined for enlargement of the spleen, and the blood of 3,294 of these was examined for the presence of malaria parasites. The results are given in Appendix C.

Whenever a child was found to have an enlarged spleen, an inquiry was made as to its recent movements. In more than half the cases, it was found that the child had either recently arrived in Calcutta, or had visited its native village within the last few months and had contracted fever there. The ideal procedure, of course, would have been to obtain the history of every child examined, but this was out of the question in the limited time available. The total number of children found to have enlarged spleens was 322 (roughly 4 per cent of those examined), and of these 167 were considered to have certainly acquired their infection from outside Calcutta. Of the remainder, in some cases it was impossible to obtain a reliable history, but it is considered that the majority of them were probably local infections. The size of spleen in the two categories is given in Table III.

TABLE III.

	INFECTIONS PROBABLY ACQUIRED IN CALCUTTA.							INFECTIONS PROBABLY ACQUIRED OUTSIDE CALCUTTA.						
	P.	1F.	2F.	3F.	4F.	U.	Total.	P.	1F.	2F.	3F.	4F.	U.	Total.
Number of spleens in each class.	111	29	12	2	1	0	155	78	28	35	18	5	3	167
Percentage	71.6	18.7	7.7	1.3	0.6	..	..	46.7	16.7	20.9	10.8	3.0	1.8	..

*N.B.*—P. = palpable; 1F., 2F., etc. = one finger-breadth, two finger-breadths, etc., below costal margin; U. = projecting to level of umbilicus.

It is noticeable that the spleens in the first category were of considerably smaller size than those in the second, 90 per cent of them projecting not more than one finger-breadth below the costal margin, as against 63 per cent in those whose infection was probably acquired outside Calcutta. This is what one would expect, as the latter category includes recent arrivals from highly malarious areas where the population would be exposed to repeated infection.

In calculating the splenic index for the various wards (Map I), the cases which are considered to have acquired their infection from outside the city have been excluded. The figures thus arrived at are throughout extremely low, the highest being recorded in Wards 21 (Ballyganj), 14 (Taltolla) and 24 (Kidderpore). In no case did the corrected index exceed 4 per cent. If all the children with enlarged spleen are included, the figure for each ward is still less than 5 per cent.

As regards the blood examinations, it was of course impossible to find out whether those children in whose blood malaria parasites were found had acquired their infection from outside Calcutta or not. The results show considerable differences in the various wards, and if we assume that the amount of movement of the population is approximately the same throughout the city, certain facts emerge with regard to the amount of malarial infection actually present. One advantage in studying the parasite indices is that we can be quite sure that we are actually dealing with cases of malaria, and not with cases which might possibly be kala-azar.

The figures indicate that the northern part of the city proper is almost entirely free from malarial infection, though figures of 2 and 3 per cent are found in the Cossipore-Chitpore added area. The south-western quarter of the city also yields very low figures. When we come to the south-eastern quarter (Ballyganj and Tollyganj) there is a distinct increase in the figures, the percentage of positive bloods being 8 and 7 respectively. There are also comparatively high figures in the central part of the city, in Wards 13 (Fenick Bazar) and 15 (Collinga). In one school in Wellesley Street (Ward 13) the parasite index was 19.3 (31 observations), whilst in another school in Bedford Lane (Ward 15) the index was 15 (40 observations). These figures are significant when we consider that in the majority of the wards the parasite index was below 5 per cent, and that in six wards it was actually *nil*. They indicate that there are definite foci of malarial infection even in the heart of the city, for the children attending the schools, especially in the case of the smaller ones, usually live in the immediate vicinity (Map II).

The total number of blood films found to contain malaria parasites was 85 (2.6 per cent of those examined). Of these 68, or 80 per cent, were malignant tertian infections, and the remainder benign tertian.

It is interesting to note that in spite of the widespread belief in Calcutta that the presence of tanks is associated with malaria, the results of the present

# MAP I.

## MAP OF CALCUTTA

The figures in brackets represent corrected splenic indices found among municipal school children in 1932.

### DISTRICT I

1. Shampukur
2. Kumartuly
3. Burtolla
4. Sukea St.
5. Jorabagan
6. Jorasanko
30. Belgachia
31. Satpukur
32. Cossipore

### DISTRICT III

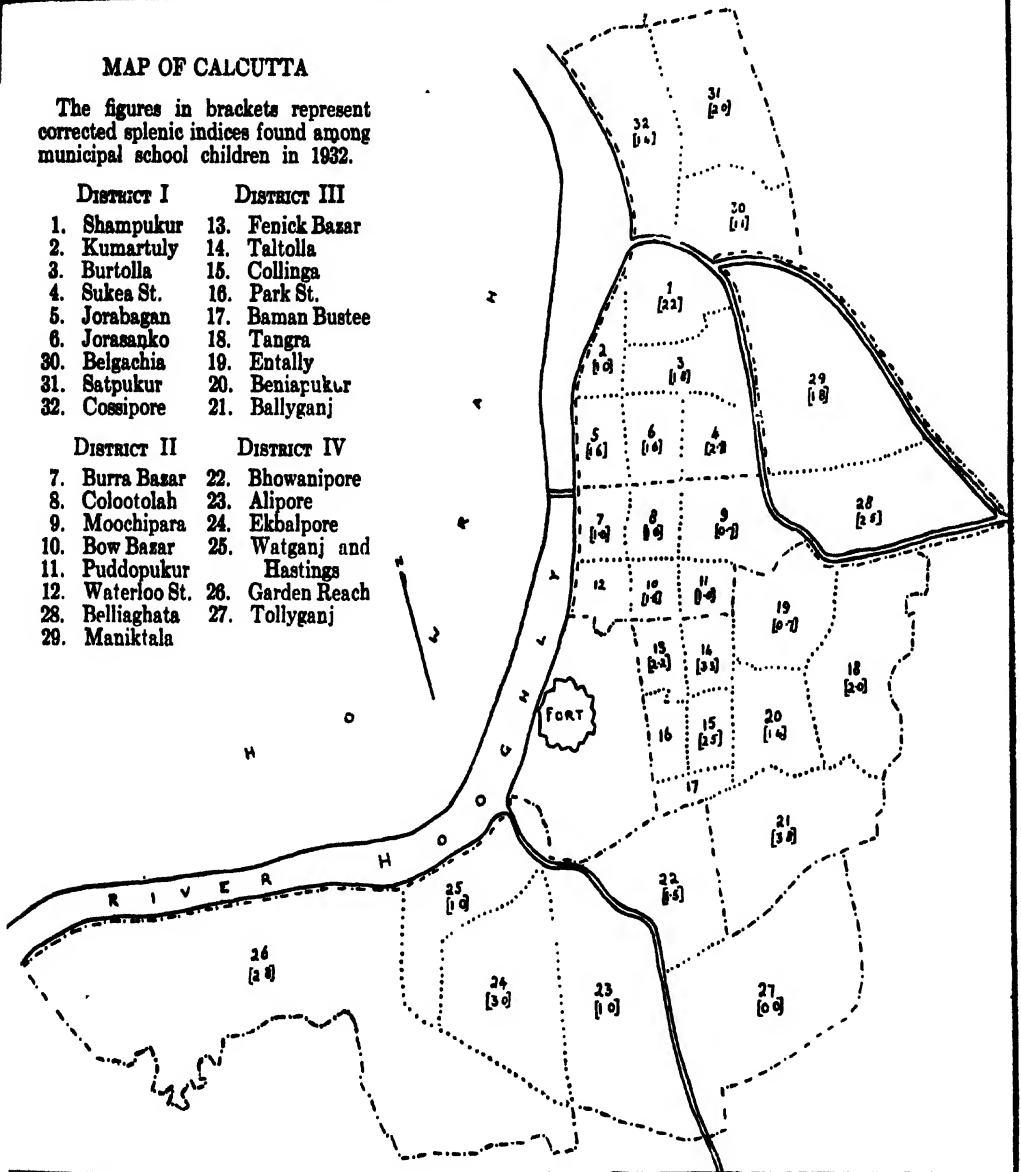
13. Fenick Bazar
14. Taltolla
15. Collinga
16. Park St.
17. Baman Bustee
18. Tangra
19. Entally
20. Beniapukur
21. Ballyganj

### DISTRICT II

7. Burra Bazar
8. Colootolah
9. Moochipara
10. Bow Bazar
11. Puddopukur
12. Waterloo St.
28. Belliaghata
29. Maniktala

### DISTRICT IV

22. Bhowanipore
23. Alipore
24. Ekbalpore
25. Watganj and Hastings
26. Garden Reach
27. Tollyganj





# MAP II

## MAP OF CALCUTTA

The figures within circles represent the parasite indices found among municipal school children in 1932

### DISTRICT I

1. Shampukur
2. Kumartuly
3. Burtolla
4. Sukea St
5. Jorabagan
6. Jorasanko
30. Belgachia
31. Satpukur
32. Cossipore

### DISTRICT III

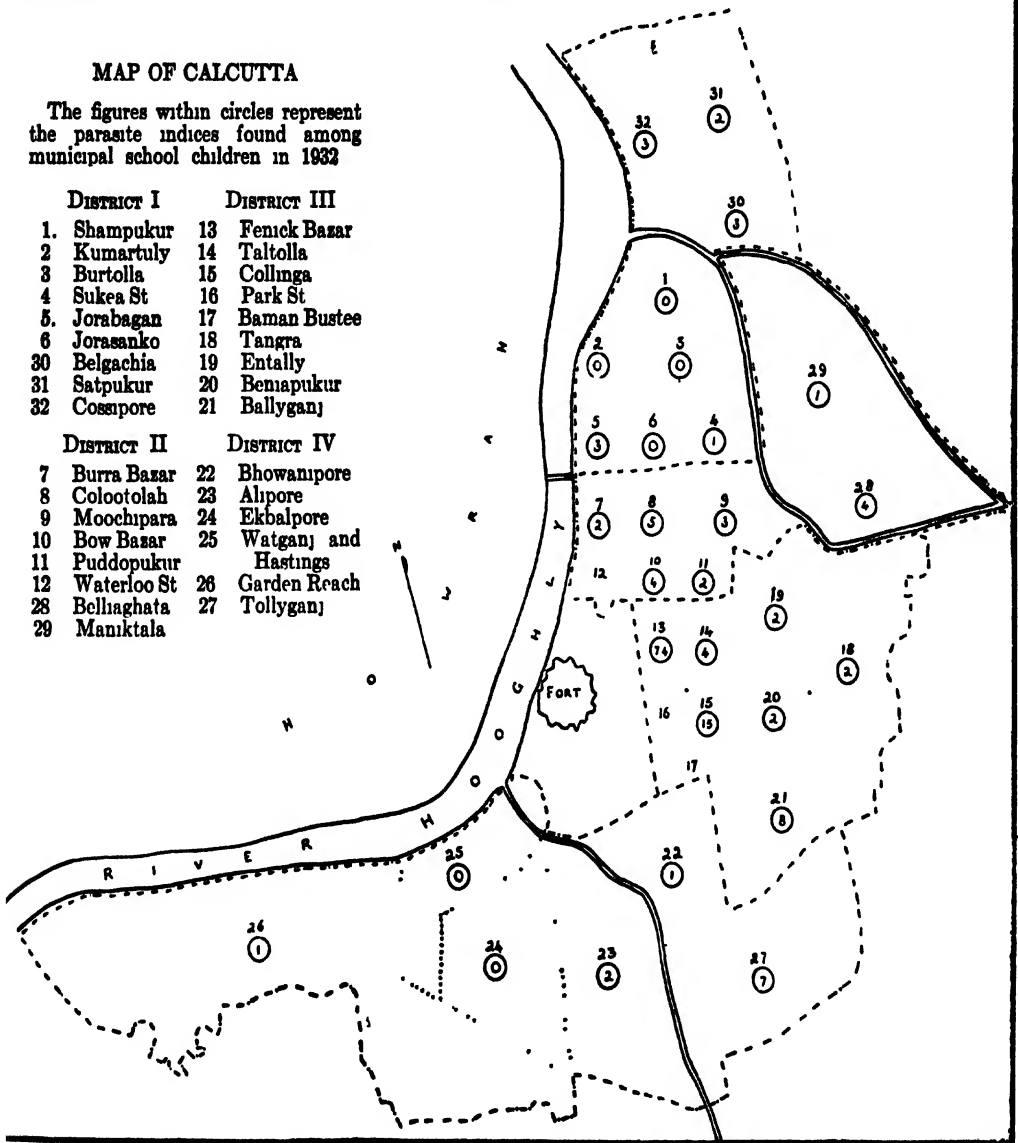
13. Fenick Bazar
14. Taltolla
15. Collinga
16. Park St
17. Baman Bustee
18. Tangra
19. Entally
20. Beniapukur
21. Ballyganj

### DISTRICT II

7. Burra Bazar
8. Colootolah
9. Moochipara
10. Bow Bazar
11. Puddopukur
12. Waterloo St
28. Bellaghata
29. Maniktala

### DISTRICT IV

22. Bhowanipore
23. Alipore
24. Ekbalpore
25. Watganj and Hastings
26. Garden Reach
27. Tollyganj





investigation show that many of the wards in which tanks are very numerous are considerably less malarious than other wards in the heart of the city. Thus Maniktala has a splenic index of 1.8 and a parasite index of 1.0, and the indices in Belliaghata, Entally and Tangra are also very low.

As has been noted in Chapter II, the careful observations of Rogers and Megaw proved clearly that there was a considerable amount of local transmission of malaria in Calcutta, in spite of the fact that there was but little evidence of the existence of endemic malaria in the city. This point was also brought out in articles by several other observers which have appeared in subsequent years, and which are quoted in the same chapter. The study of returns from the various hospitals and dispensaries in the city are of comparatively little value in judging of the extent of malaria in Calcutta, as it is impossible to tell from these whether the cases treated had acquired their infection locally or from outside. The figures from various sources are given in Appendix B. The data regarding the Calcutta Police are perhaps the most valuable, as they probably include the greatest proportion of cases of local origin. In the case of troops stationed in Calcutta, it is estimated that though locally acquired cases of malaria do occur from time to time, these do not amount to more than one or two per annum, and that the vast majority of the cases are caused by infection acquired when the troops are out at camp. It is unfortunate that the period selected for this should coincide with the most favourable season of the year for malaria transmission.

The figures for the tramway employees point to the existence of a very considerable amount of malaria in Behala, a municipality situated to the south of Calcutta. Dr. L. E. Napier, of the Calcutta School of Tropical Medicine, informs me that there have recently been a large number of cases of malaria in a community living in this locality among whom he has been making observations.

Undoubtedly a large number of these infections occurring amongst the general population of Calcutta are acquired by persons who go out of the city to malarious areas during the Pujah holidays, which occur in September or October every year. Inquiries amongst general practitioners and other medical men who have lived in Calcutta for many years, however, confirm the fact that cases of undoubted local origin occur from time to time. For instance, about the year 1925 there was a localized outbreak of malaria among the staff of the Medical College Hospital. Again, in the autumn of 1931 there were 18 cases among the staff living on the roof of Messrs. Mackinnon, Mackenzie's building in Strand Road. The records relating to the staff of the Eastern Bengal Railway employed in Calcutta show that there was an unusually large number of cases of locally acquired malaria amongst them in 1931. A practitioner living in Ward 16 (Park Street) stated that there were an exceptional number of locally acquired cases in his vicinity in the same year. There are



no municipal schools in this ward, and no children living in it were examined; but it is significant that this ward adjoins both Ward 13 and Ward 15, which contain the two schools in which the parasite index was found to be greater than anywhere else in Calcutta. On the other hand, inquiries in other parts of the city did not suggest the existence of any undue amount of malaria, and it was even stated in some cases that its prevalence had been less than usual.

Taking all the available evidence into consideration, it is clear that although endemic malaria in Calcutta generally is very slight in amount, there is an appreciable amount of local transmission of the disease, originating chiefly from cases imported from outside, and that in some parts of the city there were an unusually large number of cases in 1931.

Although local conditions may apparently remain unchanged, the prevalence of malaria, as is the case with other diseases, does not remain static, but tends to occur as it were in waves, a few years of increased malaria alternating with periods of diminished prevalence. It appears probable that the incidence of malaria in Calcutta decreased from about the year 1925 to 1930, and that it is now on the upward grade.

## CHAPTER V.

### THE ANOPHELINE MOSQUITOES OF CALCUTTA.

The following species of anophelines have been recorded in Calcutta :—

(1) *A. subpictus (rossii)*.—Stephens and Christophers (1902); James (1902); Annandale, Daniels (in Brunetti, 1907); Brahmachari, B. (1909); Paiva (1912); Crake (1916); Iyengar (1920); De (1923); Strickland and Chowdhury (1927); Senior White (1932).

This is probably the commonest species occurring in Calcutta. It is generally accepted as not being concerned in the transmission of malaria in India.

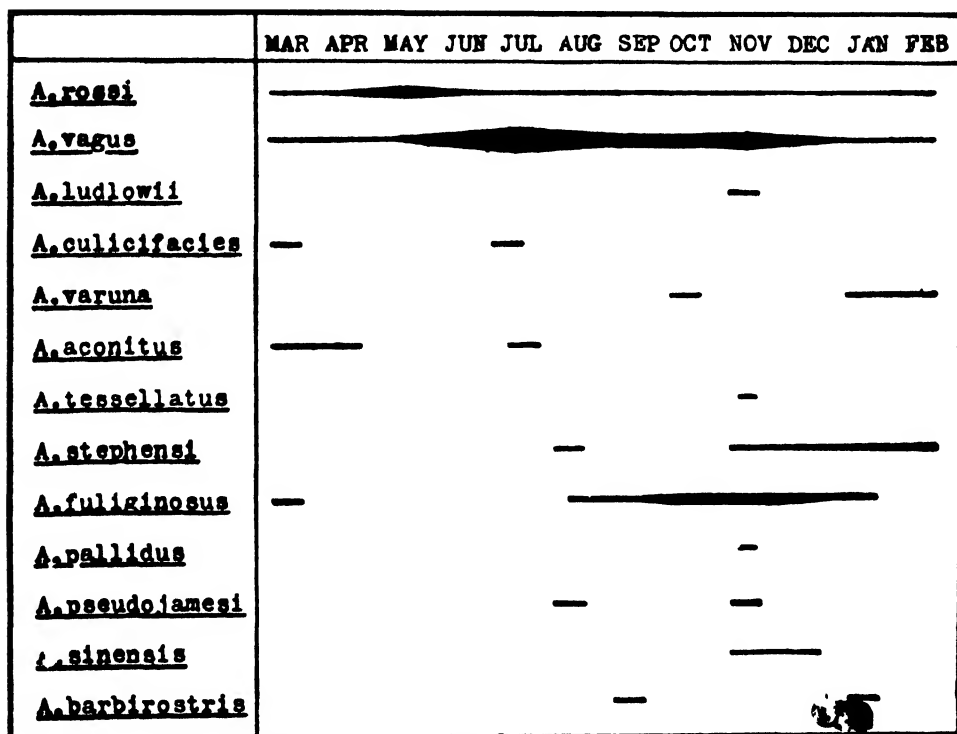


Chart illustrating seasonal prevalence of anopheles mosquitoes at Garden Reach, 1931-1932, kindly supplied by Mr. R. A. Senior White.

N.B.—Male specimens thought to be *A. minimus* and *A. listoni* were also captured in scanty numbers (3 of each species), the former in April, May and July, the latter in April, November and January. These records are considered to be doubtful.

(2) *A. stephensi*.—James (1902); Annandale, 1907 (in Brunetti, 1907); Brahmachari, U. N. (1912); Crake (1916); Iyengar (1920); De (1923); Basu (1930); Senior White (1932).

This is the notorious carrier of malaria in Bombay, Madras, Delhi and many other cities in India. It is the only malaria carrier in this country that is able to adapt itself to the conditions of life obtaining in cities, and it has been shown by Iyengar (1920) and Basu (1930) to be very prevalent even in the very centre of Calcutta.

(3) *A. fuliginosus*.—Stephens and Christophers (1902); James (1902); Daniels, 1899 (in Brunetti, 1907); Brahmachari, B. (1909); Crake (1916); Iyengar (1920); Strickland and Chowdhury (1927); Senior White (1932).

This species is commonly found in the suburbs of Calcutta. It is not considered to play any part of importance in the transmission of malaria.

(4) *A. varuna*.—Iyengar (1924); Christophers and Puri (1931); Senior White (1932). Erroneously described as *A. listoni* in earlier records :—Alcock, Adie (in Brunetti, 1907); Brahmachari, U. N. (1912); Iyengar (1920).

This species has been found infected in nature (Iyengar, 1928), and it is probable that it plays some part as a malaria carrier in the suburbs of Calcutta.

(5) *A. barbirostris*.—Annandale, 1905 (in Brunetti, 1907); Brahmachari, B. (1909); Crake (1916); Iyengar (1920); Strickland and Chowdhury (1927); Senior White (1932).

This species is found fairly commonly in the suburbs of Calcutta. It is not considered to be a malaria carrier in India.

(6) *A. hyrcanus* var. *nigerrimus* (*sinensis*).—Stephens and Christophers (1902); James (1902); Annandale, 1908 (in Brunetti, 1912); Brahmachari, B. (1909); Alcock, Daniels (in Brunetti, 1920); Crake (1916); Iyengar (1920); De (1923); Strickland and Chowdhury (1927); Senior White (1932).

This is a common species in the suburbs of Calcutta. It is not considered to be a malaria carrier in India.

(7) *A. ramsayi* (*pseudo-jamesi*).—Senior White (1932). Formerly erroneously recorded as *A. jamesi*; James (in Brunetti, 1912); James and Liston (1911); Iyengar (1920).

This species occurs fairly commonly in the suburbs of Calcutta, but it is not considered to play any part of importance in the transmission of malaria.

(8) *A. aconitus*.—Brahmachari, U. N. (1912); Alcock (in Brunetti, 1920); Strickland and Chowdhury (1927); Christophers and Puri (1931); Senior White (1932).

This species occurs in the suburbs of Calcutta, but is comparatively rare. There is no evidence that it plays any part in malaria transmission in India.

(9) *A. culicifacies*.—Brahmachari, U. N. (1912); Senior White (1932).

Though this species is an important malaria carrier in Northern India, it is too rare in Calcutta to be of any significance in transmission.

(10) *A. vagus*.—Strickland and Chowdhury (1927); Senior White (1932).

This species closely resembles *A. subpictus (rossii)*, and has probably been confused with the latter species by previous observers. It is not considered to play any appreciable part in the transmission of malaria in India.

(11) *A. ludlowii* var. *sundaica*.—This species was recorded in Calcutta by Paiva (1912) and by Brahmachari, U. N. (1912). Paiva's investigations were carried out over a period of 12 months, and the only species of anopheline recorded by him other than *A. ludlowii* was *A. subpictus (rossii)*. *A. stephensi* is not mentioned in his paper, but the breeding-places in which larvæ of *A. ludlowii* are said to have been found are those which are typically selected by *A. stephensi*. There can be little doubt that the species recorded by Paiva was really *A. stephensi*, and not *A. ludlowii*.

Annandale and Kemp (1915), speaking of the anopheline fauna of the Chilka Lake, observed 'The absence of *A. ludlowii* is somewhat remarkable, as it is the common anopheline of brackish water near Calcutta'. Sir Rickard Christophers informs me that some specimens thought to be *A. ludlowii* were sent to him by Dr. Annandale about this time, but that they all turned out to be *A. subpictus*. The observation quoted above is therefore not considered to be reliable. The authors may of course have been referring to the records of *A. ludlowii* breeding at Port Canning.

Brahmachari's record is of more significance. The tank of the Campbell Hospital, in which he recorded the presence of larvæ of *A. ludlowii* from November to February, is situated in a compound which was at that time immediately adjacent to Sealdah Station, where, as has already been stated, adult specimens of *A. ludlowii* were captured in 1931 in trains coming from Budge-Budge and Port Canning. The larvæ found in the tank by Brahmachari are very unlikely to have been those of *A. stephensi*, which does not usually breed in such a situation. Moreover, in the same paper *A. stephensi* is recorded separately as breeding in masonry cisterns. It is considered a distinct possibility that specimens of *A. ludlowii* conveyed by trains from Port Canning to Sealdah Station may have deposited their eggs in the tank of the Campbell Hospital.

As has been already noted, during the year 1931 adult specimens of *A. ludlowii* were caught at Majerhat, Sealdah, Howrah, Bracebridge and Shambazar railway stations, and also in catching stations in the Kidderpore Docks area (Senior White, 1932).

(12) *A. pallidus*.—The capture of a single adult specimen has been recorded (Senior White, 1932).

(13) *A. tessellatus*.—The capture of a single adult specimen has been recorded (Senior White, 1932).

Of the above-mentioned species, the only one which is to be seriously considered as a malaria carrier in Central Calcutta is *A. stephensi*, though *A. varuna* may play some part in the transmission of the disease in the suburban areas. The problems presented by the presence of *A. stephensi* in Central Calcutta and of *A. ludlowii* in the vicinity of the city are discussed in separate chapters.

It is noteworthy that the only published record of dissections of anopheline mosquitoes under natural conditions in Calcutta appears to be that of Stephens and Christophers (1902), who dissected 324 specimens with negative results. James (1902), who was with Stephens and Christophers in Calcutta in 1901 when the observations were made, refers to the same dissections, and says 'Practically the only species of anopheles which was present in the parts of Calcutta examined by us was *A. rossii*', so that it is almost certain that most, if not all, of the dissections made were of this species.

## CHAPTER VI.

### THE PREVALENCE OF *A. STEPHENSI* IN CALCUTTA, AND THE MEASURES NECESSARY FOR ITS CONTROL.

*Anopheles stephensi* is the only malaria-carrying mosquito occurring in India which is able to adapt itself to the conditions obtaining in cities. It is prevalent in Bombay, Delhi, Lucknow, Madras, Bangalore and many other cities. For its breeding-places it favours fresh water, preferably constantly renewed, and its larvæ are to be found in wells, cisterns (whether of iron or masonry), garden tubs, fountain basins, disused pitch barrels, improperly graded roof gutters and terraces, disused tins, earthenware and iron pots, and any receptacles used for storing water. It also breeds in any collections of water caused by leakage from a water supply system.

*A. stephensi* will breed equally well in dark places and in those fully exposed to sunshine, and in any depth of water from a fraction of an inch to hundreds of feet. For instance, its larvæ have been found in enormous numbers in the water used for keeping the surface of cement concrete wet during building construction, whilst it breeds also in deep wells, even if situated inside houses as in Bombay. It has also a wide tolerance with regard to the temperature of the water in which it breeds. It requires only the narrowest of openings in order to reach its breeding-places, and by the term 'mosquito-proof' is meant a condition where there is no aperture greater in size than the mesh of an ordinary mosquito-net. Frequent disturbance of the water, such as by dipping it out of wells or masonry cisterns, will not prevent it breeding. With rare exceptions it does not breed in tanks or ponds, and these are relatively innocuous in Calcutta as far as malaria is concerned.

*A. stephensi* is a very efficient malaria carrier in nature, and specimens caught in Calcutta have been found experimentally to be easily infected with malaria parasites.

The researches of Iyengar (1920) and of Basu (1930) have shown that this species is exceedingly prevalent throughout Calcutta, even in the most central parts of the city, and this has been confirmed by my own observations. It is interesting to note that *A. stephensi* is not found to occur under natural conditions in Lower Bengal, and its prevalence in Calcutta provides a striking instance of its extraordinary ability to flourish in artificial water collections provided by man. In an extensive survey of a large number of villages situated in the country immediately surrounding Calcutta, it was never once found (Iyengar, 1928).

Owing to the enormous increase of water connections in Calcutta in recent years, and the development of the water carriage system of conservancy, there are now very many more breeding-places suitable for *A. stephensi* than formerly. The water supply of Calcutta is twofold, consisting of filtered and unfiltered water. Filtered water is supplied under high pressure from 5 to 10 a.m. and 3-30 to 6-30 p.m. daily, and unfiltered water from 3 p.m. to 10 a.m. For the rest of the 24 hours the supply is at low pressure only. It was hoped at one time that it would be possible to provide a continuous high pressure supply of filtered water throughout the 24 hours, and to do away with the unfiltered supply; but this has been found impracticable, and the prospect that a continuous high pressure supply will ever be provided appears to be remote. Hence the necessity for the storage of water in cisterns and other receptacles must continue.

The enormous increase in the number of water connections in recent years is shown in Table IV.

TABLE IV.

*Number of connections.*

Year.	Filtered.	Unfiltered.
1870	780	0
1875	6,893	1
1880	10,284	16
1885	13,777	168
1890	16,523	437
1895	22,424	1,214
1900	25,901	3,577
1905	29,074	10,687
1910	30,959	18,882
1915	33,474	26,514
1921	37,023	31,530
1932	50,528	41,702

The distribution of the water connections in Calcutta at present are shown in Table V.

TABLE V.  
Number of connections.

District.	Wards.	Filtered.	Unfiltered.
I	1-6	17,115	15,476
	30-32	1,560	..
II	7-12	12,454	11,814
	28-29	1,560	..
III	13-21	6,790	6,015
IV	22-25, 27	10,538	8,397
	26	313	..

Another factor in the increase of the breeding-places of *A. stephensi* in Calcutta is the shifting of kitchens to the roofs of buildings, as noted by Basu (1930). This results in an increased demand for filtered water on the roof, but as the pressure of the filtered water supply is insufficient it is collected in a masonry cistern on the ground floor, and thence pumped up into an iron cistern on the roof.

We have seen that the amount of endemic malaria existing in Calcutta at the present time is very slight, but that localized outbreaks of the disease occur from time to time, originating from persons who have become infected outside the city. It is highly probable that with the increased facilities for the breeding of *A. stephensi* the local transmission of malaria will steadily increase, especially if there continues to be a high incidence of malaria in Bengal generally, as is said to have occurred in the autumn of 1931. There is a very definite danger that the local transmission of malaria may increase to a point where it becomes a factor of considerable economic importance in the life of the city, as has happened in Bombay. The resulting dislocation of business, and the possible infection of the crews of ships, would be a serious matter.

It is therefore most strongly urged that the anti-mosquito organization recently sanctioned by the Calcutta Corporation be in the first place directed against the breeding-places of *A. stephensi*. Since these breeding-places are the same as those favoured by *Aedes aegypti* (*Stegomyia fasciata*), the carrier of dengue and yellow fever, and the mosquito which causes such annoyance by biting, even in the daytime, in Calcutta, measures taken against *A. stephensi* will also be effective in the case of these diseases, and against the mosquito nuisance generally.



The measures to be adopted were laid down in detail by the present writer in his report on Malaria in Bombay (Covell, 1928). These recommendations were evolved as the result of many years' experience of attempts to control the breeding of *A. stephensi*, and are designed to overcome difficulties which have actually arisen in practice. They have been found most effective, and it is strongly urged that they be adopted in detail in Calcutta. The task is greatly lightened by the almost complete absence of wells, the existence of which in Bombay has proved a great obstacle to the work of malaria control. The principal measures recommended are as follows:—

1. As regards cisterns, the first desideratum is a detailed survey to find out the number and location of all the cisterns in Calcutta. It is of great importance that these should be clearly numbered, otherwise it is certain that some of them will be missed in the course of inspection. It is suggested that the numbering be carried out during the course of the survey, and the condition and defects of each noted. This will of course be a long and arduous task, but it is the only method of ensuring that every cistern shall be made mosquito-proof, and it should be taken up systematically ward by ward. I would here emphasize that to search for larvæ in cisterns is entirely superfluous, and a sheer waste of labour. It is proved beyond doubt that they are a major source of breeding of both *A. stephensi* and *Stegomyia*, and the only sound course is to render them mosquito-proof and therefore innocuous.

The chief points in connection with the mosquito-proofing of cisterns are given below:—

(a) All cisterns should be covered with sheet iron or reinforced concrete.

The latter is perhaps preferable in the case of very large cisterns, such as sprinkler cisterns in mills, or overhead cisterns in connection with railways, because it lasts very much longer, and because if properly constructed there is no possibility of water collecting on the top; but owing to its weight it cannot be employed unless the supporting structure of the cistern is sufficiently strong. Great care must be taken to see that the junction between the cast-iron side of the cistern and the concrete cover is made absolutely mosquito-proof.

Corrugated iron sheets should not be permitted as coverings for any cisterns. They are seldom mosquito-proof even when first applied, and are never so after a few months' wear.

(b) Water-gauges necessitating an aperture in the roof or side of a cistern should be prohibited. Two types of mosquito-proof gauges are shown in Figs. 4 and 5.

(c) Man-hole lids should be well fitting and of the pattern approved by the municipality.

The type recommended is a circular cap-cover, the rim of which fits over another rim fixed in the roof of the cistern. The hinge and hasp of the cap-cover should be placed as close to the latter as possible (Figs. 2 and 3).

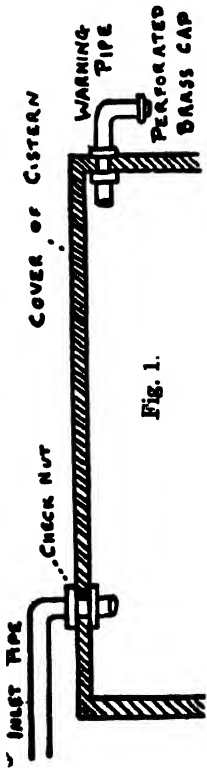


Fig. 1.

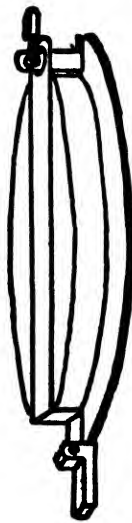


Fig. 2.

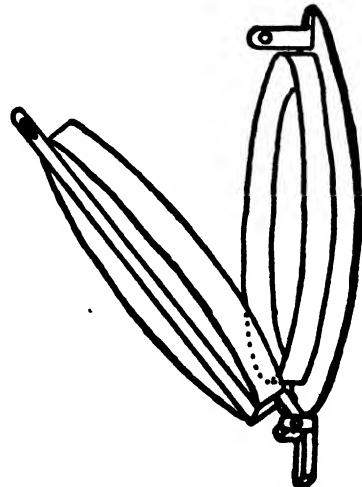


Fig. 3.

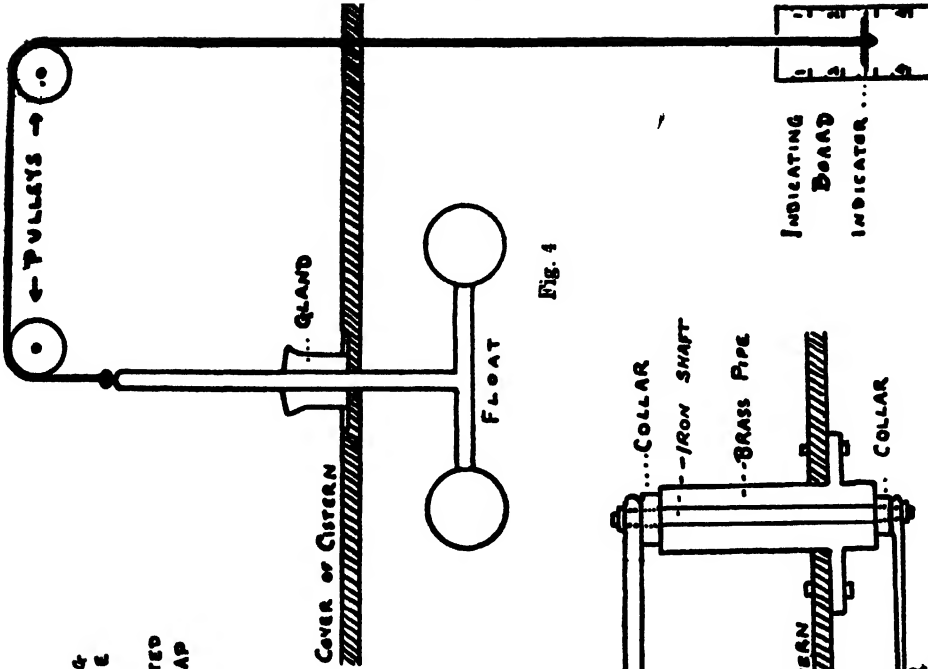


Fig. 4.

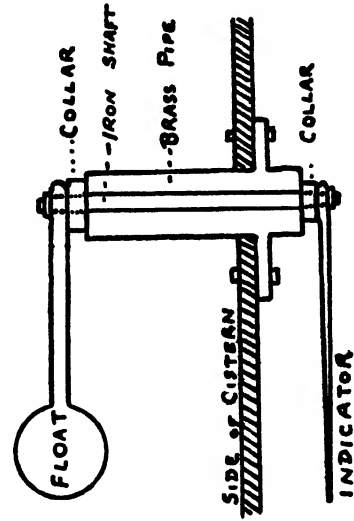


Fig. 5.

Figures illustrating points in the mosquito-proofing of cisterns.

(d) No inlet pipe should enter a cistern through a man-hole.

(e) Every inlet pipe should be provided with a check-nut (Fig. 1), or in the case of large cisterns with concrete covers with a double flange secured by bolts through the cover.

(f) The warning or overflow pipe should be protected by a standard pattern of cap approved by the municipality.

The type recommended is of brass perforated by small holes (Fig. 1). The protection of warning pipes by wire gauze is unsatisfactory, as the gauze rapidly perishes.

(g) A strong padlock of the pattern approved by the municipality should be provided for the lid of every cistern. All cistern lids should be kept closed and locked. It is a point for consideration whether the municipality or the owner should provide the lock. A penalty should be imposed in the case of any cistern found unlocked.\*

(h) Proper means of access, to the satisfaction of the Malaria Officer of the municipality, should be provided in the case of all cisterns. If a ladder is necessary this must be of iron, and should be fixed in position.

(i) Every cistern should be clearly numbered, to facilitate inspection.

(j) Every cistern should be kept in good repair.

(k) All unserviceable cisterns should be removed.

(l) Valve-boxes in cisterns should be prohibited.

(m) The Malaria Staff should inspect every cistern on a specified day in each month (i.e., first Monday in the month, etc.) and between certain specified hours. The day and hour to be notified to the owner or occupier of the house.

(n) If any cistern is found to be non-mosquito proof, the owner should be given one week's notice to repair the defect. If no action has been taken within a week, the Executive Officer of the municipality should cause the work to be carried out immediately, and recover the cost from the owner.

(o) No new cistern should be installed until it has first been inspected and passed by the Malaria Officer.

It is suggested that a model cistern be obtained of the type now used in Bombay, embodying all the above points. This would cost about Rs. 40, and could be kept as a pattern in the office of the Health Officer of the municipality.

2. As regards water-closet flush cisterns, in future the installation of cisterns with a longitudinal or circular opening in the top should be prohibited, and only those of the pattern approved by the municipality allowed. This should be of the type now used in Bombay, which is mosquito-proof.

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\* Recently in Bombay the padlocks have been replaced by strong bolts and nuts, as it has been found that this reduces the chance of the lids being opened.

3. As regards masonry cisterns (*choubachas*), it is sometimes stated that these cannot provide breeding-places because they are so frequently emptied. This is not so in many cases, for Basu (1931) found larvæ of *A. stephensi* in 286 out of 982 examined. A regulation should be made that every masonry cistern should be *completely* emptied out at least once a week. This should be done on the day of inspection by the anti-malaria staff.

4. Similar regulations should be made in respect of all other receptacles for storing water, including garden tubs.

5. One of the most important tasks of the anti-mosquito staff should be the removal of all disused tins, earthen pots and other vessels.

6. No fountains should be permitted, except those which are so constructed that no water whatever remains when the fountain has ceased playing.

7. Stand-pipes should in every case be provided with a cement drain connected with the main drainage system.

8. Road-watering hydrants should in future be provided with a cover which has no aperture in it. I would suggest also that a type should be designed the cover of which does not open quite as far as a right angle. This would then automatically close when not in use.

9. Water used to keep cement concrete wet during building construction should be treated with sufficient saponified cresol to make it just milky. Receptacles used for soaking bricks used in building construction should be emptied daily after use and turned upside down. Alternatively, saponified cresol might be added to the water.

10. Yards containing machinery, scrap-iron, etc., should be levelled and adequately drained, and the machinery, etc., so stacked as to provide no hollows in which rain water may collect.

11. In the case of all new buildings the proper grading of terraces and roof-gutters so that no water can collect should be insisted on.

12. The presence of discarded tins, earthenware vessels, etc., on roofs or in yards or compounds, found to contain mosquito larvæ, should constitute an offence punishable by law.

13. Fire-buckets should be filled with sand, or if water is used cresol should be added to it.

### **Anti-Malarial Legislation.**

It appears from a study of the Calcutta Municipal Act that the Corporation has the power of enforcing all or nearly all of the recommendations above put forward. Power of entry is secured under Section 507, and under Section 510 power is given to execute necessary works in the event of requisitions not being complied with.

It is important that amendments should be introduced where necessary to cover all the points which have been raised, such as for instance the provision of proper means of access to cisterns.

Schedule XVIII is of great importance as regards anti-malarial measures, and extracts from it are given below.

*Schedule XVIII.—Rules for the inspection and regulation of land and buildings.*

1. The Corporation may cause any building or other premises to be inspected for the purpose of ascertaining the sanitary condition thereof.

7. (1) When

(a) any well, pool, ditch, tank, pond, pit or marshy or undrained ground, or

(b) any cistern, reservoir or water-butt or any other receptacle or place where water is stored or accumulates, or

(c) any waste or stagnant water, whether within any private enclosure or not,

appears to the Corporation to be or likely to become injurious to health or offensive to the neighbourhood or in any other respect a nuisance, they may, by written notice, require the owner or occupier of the land or building to which such well, pool, ditch, tank, pond, pit, ground, cistern, reservoir, water-butt, receptacle, place or water pertains, to cleanse or fill up the same with suitable material or to drain off or remove water therefrom or to take such other order therewith as the Corporation may deem necessary.

(2) Where, in the opinion of the Health Officer, such well, pool, ditch, tank, pond, pit, ground, cistern, reservoir, water-butt, receptacle, place or water is or is likely to become a breeding-place for mosquitoes, he may enter upon the premises to which it pertains and take such steps as he thinks proper to cleanse the same.

(3) If the Corporation, in exercise of the powers conferred by Section 510, execute any work referred to in a notice issued under sub-rule (1), and if the person liable to pay the expenses of such work fails to pay the same, the Corporation may, until such expenses are paid :—

(i) lease any part of the land used in connection with the said well, pool, ditch, tank, pond, pit, cistern, reservoir, water-butt, receptacle, place or water, or any part of the said ground, as the case may be, or

(ii) retain possession of the same, or the site thereof, and utilize it for public purposes.

(4) If the said expenses be paid by an occupier of land, he may, in the absence of any agreement to the contrary, deduct the same from any rent due to the owner of the land.

8. On receipt of a written report from the Health Officer of the existence of a serious nuisance likely to affect the public health or to prove offensive to

the neighbourhood, the Executive Officer may take immediate action for the abatement or removal of such nuisance.

9. (1) The Corporation may by a general order, or by an order to affect such portion of Calcutta as may be specified therein, prohibit—

(a) the making of excavations for the purpose of taking earth therefrom, or of storing rubbish or offensive matter therein, and

(b) the digging of cesspools, tanks, ponds, wells or pits, without the special permission of the Corporation.

(3) No person shall make any excavation referred to in clause (a) of sub-rule (1), or dig any cesspool, tank, pond, well or pit, in contravention of any such order.

(4) If any such excavation, cesspool, tank, pond, well or pit is made or dug after the publication of any such order and without the permission required thereby, the Corporation may, by written notice, require the owner or occupier of the land on which the same is made or dug to fill it up with earth or other material approved of by the Corporation.

Specimens of a number of forms in use by the Bombay Municipality, which may be found useful as models, are given in Appendix D.

## CHAPTER VII.

### THE 'LUDLOWII PROBLEM', AND THE MEASURES ADVOCATED TO DEAL WITH IT.

*A. ludlowii*\* is one of the most efficient malaria carriers known, and there are many records of virulent outbreaks of the disease due to its presence.

It was first incriminated as a vector by Christophers (1912), who showed that it was responsible for the transmission of malaria in the Andamans. It occurs on the coast of Burma in places where malaria is rife (e.g., Kyaukpnyu and Akyab), and is probably the chief malaria carrier in this region. It is also the principal vector in the coastal zones of the Federated Malay States and of the Dutch East Indies.

It is probable that this species was responsible for the epidemic which occurred at Port Swettenham in the F. M. S. in 1901, recorded by Watson (1911). Hacker (1921) gives an account of a virulent outbreak of malaria due to the invasion of *A. ludlowii* in certain coconut estates on the banks of the Perak River (F. M. S.) in 1918.

*A. ludlowii* was also the cause of an epidemic at Belawan in the Dutch East Indies during the construction of the ocean harbour, where more than 24 per cent of the specimens collected in certain coolie sheds were found to be infected (Schüffner and Hylkema, 1922). There are numerous other instances of severe outbreaks in the Dutch East Indies in which this species was proved to be the carrier.

A virulent outbreak of malaria due to transmission by *A. ludlowii* was observed by the writer in the Andamans in 1926. A body of Karen coolies had been landed on an uninhabited island to fell timber, and the whole force was prostrated by a virulent outbreak of malaria, so much so that the work had to be abandoned. The only anopheline present was *A. ludlowii*, and 97 specimens were caught in 20 minutes in one of the coolie huts (Covell and Bailey, 1927).

In the epidemic at Budge-Budge in 1930, an infection rate of 23·4 per cent was recorded in this species (Iyengar, 1931d).

*A. ludlowii* is a brackish water breeder, but the percentage of salt found in its breeding-places varies considerably in different instances. It has been found breeding profusely in water containing from 0·03 to 1·8 per cent of sodium chloride, and larvæ have also been found in concentrations approaching

\* The species referred to as *A. ludlowii* throughout this paper is *A. ludlowii* var. *sundaica* Rodenwaldt. According to King (1932) this is probably a distinct species, in which case the correct name would be *A. sundairus*.

3 per cent. Opinions vary as to the optimum amount of salinity for breeding. Thus, Rodenwaldt and Essed (1925) in the Dutch East Indies considered that from 1.2 to 1.8 per cent was the most favourable concentration, whilst Iyengar (1931*d*) at Budge-Budge found the optimum amount to be 0.15 to 0.25 per cent. Van Breemen (1919) records *A. ludlowii* as breeding freely in concentration of 0.03 to 2 per cent, Schüffner and Hylkema (1922) in from 0.04 to 1.25 per cent, Christophers (1912) in a single observation in 0.4 per cent. *A. ludlowii* has also been found on occasions breeding in fresh water, but this is unusual and probably always temporary. Thus on one occasion in an inland locality in the Dutch East Indies, Schüffner was experimenting with specimens of *A. ludlowii* collected in the coastal zone. Several escaped, and deposited their eggs in a fresh water pond, and continued to breed there for some generations. However, after some time the breeding ceased, and the species has never again been found there (Swellengrebel and S. de Graaf, 1919).

*A. ludlowii*, unlike the majority of malaria-carrying species of mosquitoes, can tolerate a considerable degree of organic pollution in its breeding-places. Indeed, according to Rodenwaldt and Essed (1925) it actually favours breeding-places which are heavily polluted. These observers state 'Its cardinal condition is the presence of decaying matter. Thus, it is not hampered by the breeding-places being strongly polluted with urine and with faeces. It may be that the fish ponds even at the said salt percentage (1.2 to 1.8 per cent) are offering the best conditions for breeding, because there the consequences of the decay of the algæ are developing best'. Iyengar (1931*d*) observed that *A. ludlowii* at Budge-Budge bred in water with some organic contamination. He found that every one of the ponds in which a salinity of more than 0.1 per cent occurred in combination with a trace of nitrites contained larvæ of *A. ludlowii*. Where the salinity was more than 0.1 per cent, but nitrites were absent, *A. ludlowii* occurred very rarely. He concluded that a combination of the salinity factor with the presence of a small amount of organic contamination seemed to be the optimum condition for the breeding of *A. ludlowii* in this area. More recently, however, in a number of instances, the larvæ of this species have been found in the Lower Bengal area in water free from any trace of nitrites. As will be discussed later, the question of organic pollution and the breeding of *A. ludlowii* has an important bearing on the problem of malaria in the vicinity of Calcutta.

*A. ludlowii* favours breeding-places in which floating algæ (*Enteromorpha*, *Cladophora*, *Cyanophyceæ*) are present. The observations of the officers of the Bengal Health Department have shown that *A. ludlowii* is most frequently found in association with *Oscillatoria germinata*, *Lyngbya confervoides*, and *Ædogonium* sp. It will also breed however in water in which no algæ are present, or at any rate none visible to the naked eye. In Belawan



it was observed that the larvæ were at first abundant in breeding-places without algæ, but that later on when algæ appeared in certain water collections, larvæ of *A. ludlowii* were exclusively found in the latter (Schüffner *et al.* 1919). This agrees with the present writer's observations in the Andamans. Schüffner and his co-workers also observed that in the coastal zone the larvæ were found in small pools, whether covered with grass, rushes or other plants or free from coarse vegetation; also in marshy ground and meadows, in small depressions formed by the footprints of men and animals.

*A. ludlowii* breeds in pools which are reached by the spring tides only, and in pools formed by the percolation of salt water through embankments. It prefers also breeding-places exposed to sunlight. 'Where the tide runs in and out freely, as in mangrove swamps, it appears to be absent; but if human intervention, by the construction of bunds, locks, etc., creates obstacles to the tidal water, the conditions change; grass begins to grow between the roots of the rhizophores, and larvæ of *A. ludlowii* soon begin to appear. A place comparatively healthy before the commencement of the operation is thus rendered highly malarious' (Swellengrebel and S. de Graaf, 1920). It has been observed that when silt-laden water is led into the breeding-places of *A. ludlowii* the larvæ disappear. This has an important bearing on the question of control measures.

As regards the habits of the adults, *A. ludlowii* is a 'domestic mosquito' showing a marked preference for the blood of man. It bites chiefly in houses at night, though it has also been known to bite in the open in the daytime on cloudy days during the rains. It is a voracious feeder and a strong flier, and it has been known to cover distances of as much as three miles in its search for blood. If a blood meal is available near at hand, however, it is not normally found at a distance of more than half a mile from its breeding-place.

It appears certain that the presence of *A. ludlowii* at Falta, Budge-Budge, Chengail and Ulubaria represents a recent colonization by this species. Its patchy distribution, its intensive breeding in small areas where its presence had never before been observed, and the virulence of the outbreaks of malaria in these areas, where the incidence of the disease had hitherto been negligible, all lend support to this view.

Whether other foci of *ludlowii* breeding nearer Calcutta will be formed in the near future, it is impossible to say. From the numbers captured at Shamnagar, 19 miles north of Calcutta, it is practically certain that the species was breeding there in 1930. But in spite of careful search no larvæ were discovered and neither larvæ nor adults have been captured there since that date. It is also of interest that no larvæ have been found at Ulubaria since August 1931. In neither of these places have any anti-larval measures been carried out, and it is possible that breeding may also die out in other foci which have recently been colonized.

It is difficult to say why *A. ludlowii* has established breeding-places up the Hooghly during recent years. In the case of the outbreak on the banks of the Perak River, the extension of *ludlowii* breeding followed a prolonged and unprecedented period of drought, and Hacker (1921) suggested that the pools present may have become more saline through evaporation, and have thus become more favourable as breeding-places. No such factor, however, can be adduced in the present instance, and there seems to have been no recent change in the local conditions in the areas where *A. ludlowii* has recently appeared in Lower Bengal.

It seems probable that for some reason the breeding of *A. ludlowii* in the Sunderbans area has been exceptionally profuse in the last few years, and that therefore larger numbers of this species than usual have been transported by country boats up the Hooghly and other channels of the delta, and have deposited their eggs in various localities. The reason for this may be that large areas of mangrove swamps have recently been cleared in the Sunderbans, but from the fact that the breeding of *A. ludlowii* has also become more extensive in the vicinity of Port Canning and Hasnabad it appears more probable that there has been a general increase of the species in the Lower Bengal delta, possibly as the result of exceptionally favourable meteorological conditions. It is noteworthy that 1931 has been an unusually bad malaria year in many parts of Bengal, and it is possible that conditions have been exceptionally favourable for mosquito life in general.

A parallel instance of a serious outbreak of malaria due to the conveyance of mosquitoes by boats is provided by the appearance recently of the well-known African malaria carrier, *A. gambiae*, at Natal, Brazil. This species, which had never before been recorded in the New World, was found breeding in large numbers near the anchorage of ships which bring the mail from Dakar on the African coast. The outbreak of malaria was unusually severe, and the infection rate among *A. gambiae* was no less than 62·8 per cent (Davis, 1931).

We must now consider the possibilities of *A. ludlowii* establishing itself in the most important areas which would affect Calcutta itself. These are (1) the swamp near Majerhat, and (2) the Salt Lake area.

As regards Majerhat, extensive breeding of *A. ludlowii* in this locality would be of grave danger to the docks and to the shipping berthed there. The Majerhat swamp is gradually being filled with silt from the bed of Tolly's Nullah, but it will take at least five years to complete the work. Fortunately, the salinity of the water in the swamp is considerably lower than the optimum concentration for the breeding of *A. ludlowii*, analysis of samples taken at various points during 1931 showing a salt percentage of from 0·006

to 0.025.\* Furthermore, adult specimens of *A. ludlowii* must have been conveyed to Majerhat Station in the trains from Falta in considerable numbers before their presence was detected in August 1931; yet no larvæ of this species were ever found in the water collections near the station. For these reasons I do not think that the breeding of *A. ludlowii* in the Majerhat swamps is likely, especially if the breeding-places at Falta are efficiently controlled.

As regards the Salt Lake area, the position is rendered grave by the silting up of the Bidyadhari River, which has only occurred very recently. Until this happened the lake was tidal, but now this is no longer so, and it is even found necessary at times to drain water off from it into the canal which runs along its northern border. The sewage outfall from Calcutta is discharged into the Bidyadhari, and the silting up of this river means that a certain amount of organic pollution will occur in the lake. This will be no deterrent to the breeding of *A. ludlowii*, and may in fact be favourable to it, for as we have seen the species can tolerate a high degree of pollution, and some observers have even stated that it prefers breeding-places which are thus contaminated. There is however one factor which is favourable, and that is that at any rate on the side of the lake nearest Calcutta there are no small pools, and I understand that, in the rainy season especially the whole lake consists of enormous sheets of water; whereas *A. ludlowii* as a rule prefers small collections of water as breeding-places. Nevertheless, the situation requires careful watching, and the provision of an organization prepared to apply rigid control measures should occasion arise.

Any engineering project which will provide for the opening up of the channel of the Bidyadhari River and the restoration of tidal influence in the Salt Lake basin should be regarded as an important anti-malaria measure.

#### RECOMMENDATIONS FOR THE CONTROL OF *A. ludlowii*.

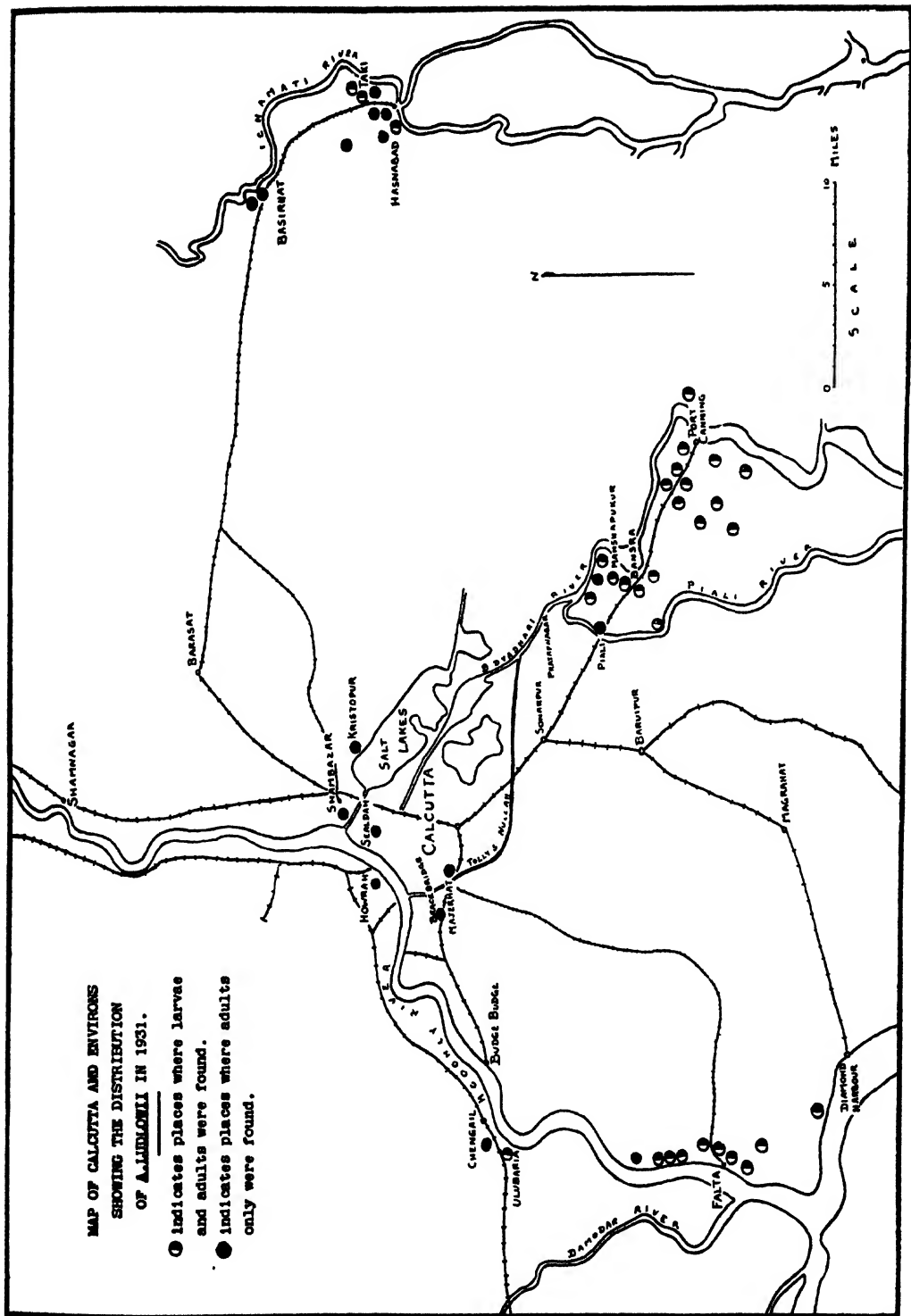
1. *Inspection of country boats.*—There are two directions from which an extension of the breeding foci of *A. ludlowii* towards Calcutta is possible, as the result of conveyance by boats. These are (i) up the Hooghly river from Midnapore and the Sunderbans, and (ii) up the Bidyadhari River and Kristopur Canal from Port Canning and its vicinity.

It has been observed that no adult mosquitoes are to be found in boats which have just entered Tolly's Nullah by passing under the Port Trust Railway bridge. This is so low that the cover of each boat has to be removed before it can pass under the bridge. It has been shown how important is the carriage of *A. ludlowii* by boats, and how this has led to the formation of new breeding

\* These figures were obtained in August 1931. Further analyses of 37 water collections in Majerhat swamp were made in March 1932. Of these, 25 yielded figures of less than 0.025 per cent, whilst 12 were greater than 0.025, but less than 0.50 per cent.

MAP OF CALCUTTA AND ENVIRONS  
SHOWING THE DISTRIBUTION  
OF A. JUDINOWII IN 1931.

- ① indicates places where larvae and adults were found.
- indicates places where adults only were found.





foci followed by severe outbreaks of malaria. It is therefore suggested that all country boats shall be boarded (a) at Falta on the Hooghly, and (b) at Pratapnagar Police Station on the Bidyadhari River, and the boatmen required to remove the cover of the boat for half an hour immediately before proceeding upstream. The inspector would then issue a signed certificate to the effect that this has been done, and a penalty should be inflicted on any boatman discovered further upstream without such a certificate in his possession.\* In the event of heavy rain, the covered-in portion of the boat might be treated with an anti-mosquito spray as an alternative measure.

I understand that the number of country boats coming up the Hooghly and Bidyadhari rivers does not usually exceed 12 per diem in each case. It would of course be necessary to have a regulation that every country boat coming up the Hooghly or Bidyadhari should tie up at Falta or Pratapnagar as the case might be.

The uncovering of the boats at Falta should very greatly minimize any danger of specimens of *A. ludlowii* being carried to places further up the Hooghly or down Tolly's Nullah; whilst the same procedure at Pratapnagar would protect the Salt Lake area from invasion by insects carried up the Bidyadhari from Port Canning and the adjacent villages, or up the Piali River.

The proposal appears to be quite practicable as regards Pratapnagar, but it is realized that it may prove impossible to compel boats coming up the Hooghly to put in at Falta, where the river is about one mile in width. If it can be done, however, it is a measure of sufficient importance to merit serious consideration.

2. *Control measures at Falta.*—The bunds between the four large borrow-pits close to Falta Station should be completely removed, so that they may form one large tank, and the edges of the tank should be kept clean cut and steeply graded. This work should be commenced at once, and completed before the end of May. The water in the tank should also be kept free from vegetation. It will probably be necessary to continue to use paris green on this tank, for the removal of the sluice gates to let the water of the Hooghly freely into it would do great damage to the rice crop, and could hardly be enforced.

3. *Control measures at Budge-Budge.*—The flood and flush scheme for southern Budge-Budge recently submitted by the Chief Engineer of the Bengal Health Department should be put in hand immediately. This scheme involves the connecting up of the tanks in this area, and the introduction into them of silt-laden water from the Hooghly, and its completion will greatly diminish the number of potential breeding-places of *A. ludlowii* in this area, and hence will cut down the present recurring expense of control with paris green. It is

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\* Or, it might be found sufficient to refuse entry into canals and harbours unless the certificate could be produced.

noteworthy that the recent virulent epidemic of malaria in Budge-Budge was almost entirely confined to the southern area, and that the northern area, which is already under the operation of a similar scheme, was only slightly affected. Meanwhile, it will be necessary to continue the temporary control measures at present in operation.

4. *Control measures at Port Canning.*—The presence of *A. ludlowii* at Port Canning was recorded as long ago as the year 1909, and it is possible that this species has bred there ever since the construction of the protective bund. But there has evidently been a large increase of breeding recently, judging from the fact that there was a sharp outbreak of malaria there in the autumn of 1931. It is probable also that the recent finding of breeding-places of *A. ludlowii* along the course of the river as far up as Piali represents a recent invasion from the focus at Port Canning. The water collections behind the bund form ideal breeding-places for *A. ludlowii*, and extend right up to the railway station. As this is the place from which *A. ludlowii* may be conveyed up the Bidyadhari and Kristopur Canal to the Salt Lake area it is of great importance that these breeding-places should be controlled.

The large borrow-pit beside the station requires regular weekly treatment with paris green. The numerous small borrow-pits along the inner aspect of the embankment on the other side of the station (in which *A. ludlowii* was breeding freely at the time of my visit) should be connected up so as to form one collection of water, and be similarly treated. In future, any earth necessary for repairing the bund should be taken from ground lying on the outside of the bund only. The numerous small pools in and around the village should be filled in from one tank, which should be made to have a clean and steep edge. Control of the breeding of *A. ludlowii* at Port Canning does not appear to be a difficult proposition. It would probably cost about Rs. 2,000 per annum.

5. *Control measures at Hasnabad.*—There is no boat traffic in the direction of Calcutta from this region, but specimens of *A. ludlowii* have been caught at Shambazar Station, the terminus of the Barasat-Basirhat Railway, and there is thus a possibility that the species might be introduced into the northern end of the Salt Lake. The railway runs close to the border of the lake, and there is possibility that mosquitoes might reach the lake from Bagniati Station, where carriages are brought for repair. Hence it is advisable to carry out control measures in this area also. I had no time to visit the Hasnabad area, but I understand that the problem there is similar to that at Port Canning.

6. *Staff and appliances.*—I cannot speak too highly of the work of the Bengal Health Department, aided by the loyal co-operation of the Malaria Staffs of the Garden Reach Anti-Malaria Association, and of the Bengal-Nagpur and Eastern Bengal Railways, in dealing with the situation up to date. But

the paucity of staff has been keenly felt, as was noted at successive meetings of the special committee convened by the Government of Bengal to advise on the situation. It is to be hoped that new foci of *ludlowii* breeding will not be established in the future; but should this occur, it will be quite impossible for the Health Department to continue to cope with the situation with its present staff. Trained men cannot be produced at a moment's notice, and I therefore recommend the *immediate* creation of two more survey units similar to the one now operating, i.e., each consisting of a controlling officer and five sanitary inspectors. The cost of each unit will be about Rs. 12,000 per annum. It should also be clearly understood that this organization will be necessary for an extended period, and I would strongly urge that provision be made for it to be maintained for a period of at least five years in the first instance. This should not be regarded as an unprofitable expenditure of public money, but as a wise scheme of health insurance. Even should *A. ludlowii* recede during the next few years, there are many important malaria problems to be solved in Bengal on which the anti-malarial staff could be profitably employed. For instance, it is not yet known for certain which species of mosquito is the chief carrier in Lower Bengal, or which species has been responsible for the great increase in the incidence of malaria in parts of the Magrahat area since the introduction of the drainage scheme.

In addition to the immediate increase in staff indicated above, I recommend that a sum of Rs. 10,000 be set aside for the purchase of anti-mosquito appliances and materials, in addition to the sum sanctioned for this purpose in 1931. It may not be found necessary to use this sum, but it should be available for instant use in case of emergency. I would also advise the immediate purchase of 12 rotary blowers and 6 mixers for use with paris green. One ton of soft stone powder and a supply of paris green should be held in reserve.

7. *The Malaria Advisory Committee.*—The valuable work done by the Committee convened by the Government of Bengal to watch the situation and co-ordinate control measures has already been referred to. It is strongly advised that this committee shall continue to meet from time to time. An officer of the Malaria Survey of India could no doubt be deputed to attend a meeting of the committee on any special occasion, should the committee so desire, as has been arranged in the case of the Malaria Advisory Committees at Vizagapatam and Bombay.



## CHAPTER VIII.

### SUMMARY AND CONCLUSIONS.

1. The amount of endemic malaria existing in Calcutta at the present time is very slight.

2. Nevertheless, transmission of malaria does occur in Calcutta, probably originating chiefly from imported cases, leading to localized outbreaks in various parts of the city. This has probably been the case from the earliest times, though records prior to the year 1900 are unreliable. There is definite evidence that there has been a considerable amount of locally acquired malaria at various times during the past 30 years.

3. *Anopheles stephensi*, which is the only malaria-carrying mosquito in India capable of adapting itself to the conditions obtaining in cities, is undoubtedly the transmitting agent of the disease in Central Calcutta. This species is breeding freely throughout the city, chiefly in cisterns and other receptacles for storing water. Except on rare occasions it does not breed in tanks, which are probably of little importance as regards malaria in Calcutta. The breeding-places of *A. stephensi* have become very much more numerous in recent years with the development of the water-carriage system of conservancy and the enormous increase in the number of water connections.

4. This being so, the numbers of this species in Calcutta are probably greater than they have ever been before, and they are increasing year by year. If the breeding-places are allowed to continue in their present condition, it is exceedingly probable that in the future the prevalence of locally acquired malaria in Calcutta will be increased, and will form a factor of definite economic importance in the life of the city.

5. The obvious remedy is to institute a vigorous campaign directed against the breeding-places of *A. stephensi*. The nature of these breeding-places, which are exclusively man-made, are well known. The measures required were laid down in detail by the writer in his report on Malaria in Bombay, 1928, and it is recommended that similar measures be carried out in the case of Calcutta. It is strongly urged that the efforts of the anti-mosquito organization recently sanctioned by the Corporation of Calcutta be directed in the first place against the breeding-places of *A. stephensi*. Since these breeding-places are the same as those favoured by *Aedes aegypti* (*Stegomyia fasciata*), the carrier of dengue and of yellow fever, and the most vicious biter of all the mosquitoes of Calcutta, the measures recommended will be effective against these diseases as well as malaria, and will tend to reduce the general 'mosquito nuisance'.

6. Breeding-places of *Anopheles ludlowii*, another well-known malaria carrier, have been found at various points within 12 to 20 miles of Calcutta, and the presence of this mosquito has been responsible for virulent outbreaks of malaria at Budge-Budge, Chengail and Falta withip the last two years. There is strong evidence that this species has only recently been introduced into these localities. Adult specimens of *A. ludlowii* have been caught at Majerhat, Bracebridge, Kidderpore, Howrah, Scaldah, Shambazar, Kristopur and Shamnagar, to which places they have been conveyed by trains or country boats. There is an extensive swamp at Majerhat, in close proximity to the docks, in which *A. ludlowii* might breed if the conveyance of this species by trains from Falta were allowed to continue. There is also a definite danger that *A. ludlowii* may gain a footing in the Salt Lake area to the east of the city. The Salt Lakes, which were formerly tidal and thus unsuitable as breeding grounds of *A. ludlowii*, have in recent years become non-tidal, owing to the silting up of the Bidyadhari River.

7. Hitherto the situation as regards *A. ludlowii* has been kept under control by the Bengal Health Department, with the co-operation of the Garden Reach Anti-Malaria Association, and of the anti-malarial staffs of the Bengal-Nagpur and Eastern Bengal Railways. In order to cope with possible further developments it is of vital importance that the staff of the Bengal Health Department be immediately increased by the addition of two more survey units, and that it should be empowered to institute control measures at any point where these may be found necessary. It is recommended that a sum of Rs. 10,000 be set aside for control measures, in addition to the sum sanctioned in 1931, to be used should the need arise. A dozen rotary blowers, and six mixers for paris green should be purchased immediately, and one ton of soft stone powder and a supply of paris green be held in readiness.

8. Since it has been proved that country boats play an important part in the conveyance of *A. ludlowii* and the formation of new breeding foci by this species of mosquito, it is suggested that all such boats be inspected at Pratapnagar on the Bidyadhari River and at Falta on the Hooghly, and their covers temporarily removed. This procedure would greatly minimize the danger of *A. ludlowii* being carried into the Salt Lake area from the direction of Port Canning, and up the Hooghly from the Sunderbans and Midnapore.

9. The flood and flush scheme for South Budge-Budge recently submitted by the Chief Engineer, Bengal Health Department, which has for its object the introduction of silt-laden water from the Hooghly into the breeding-places in that area, should be put in hand immediately.

10. Control measures should be rigidly carried out at Falta, Port Canning and Hasnabad, where the breeding of *A. ludlowii* is still going on.

11. Any engineering scheme designed to restore tidal influence in the Salt Lakes should be regarded as an important anti-malaria measure.

12. It is suggested that a spleen census of municipal school children be made annually in the same month each year, and the results recorded in the annual report of the Health Officer, Calcutta Corporation. It is also suggested that blood films be taken from 100 children in each ward of the city annually, and examined for the presence of malaria parasites.

13. It is recommended that the medical officers in charge of dispensaries and hospitals in Calcutta be asked to keep a separate record of malaria cases considered to have acquired their infection locally, and that the Police and Military and Railway authorities be asked to furnish similar information. The data thus obtained should be recorded in the annual reports of the Health Officer, Calcutta Corporation. These data, together with the records of spleen and blood examinations of children suggested in the preceding paragraph, would form a valuable index as to the incidence of malaria in different parts of the city from year to year.

14. It is strongly recommended that the Malaria Sub-Committee appointed by the Government of Bengal should continue its work. It is suggested that the Director, Malaria Survey of India, or an officer delegated by him, should be a member of this sub-committee.

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**APPENDIX A.**  
**CLIMATIC DATA FOR ALIPORE (CALCUTTA).**

TABLE I.  
*Temperature (in degrees Fahrenheit).*

			MEANS FOR OBSERVATIONS. HOURS, 1901—1920.			NORMAL MONTHLY TEMPERATURES.		
			6 a.m.	2 p.m.	10 p.m.	Max.	Min.	Range.
		Monthly normals derived from 24 hourly tabulations of dry bulb thermograms, 1901—1920.						
January	..	64·8	57·9	73·9	62·0	77·3	55·5	21·8
February	..	69·3	62·2	78·3	66·7	82·0	60·0	22·0
March	..	78·5	71·0	87·7	75·6	90·9	69·3	21·6
April	..	84·2	77·4	93·5	80·7	95·6	75·7	19·9
May	..	85·8	80·0	94·1	82·3	94·5	77·5	17·0
June	..	84·9	81·0	90·1	82·7	91·5	78·8	12·7
July	..	83·6	80·7	87·3	82·1	88·4	78·6	9·8
August	..	83·0	80·2	86·4	81·8	87·6	78·4	9·2
September	..	82·6	79·5	86·5	81·1	88·0	78·0	10·0
October	..	79·8	75·7	85·1	77·5	87·2	74·3	12·9
November	..	72·0	66·2	79·6	69·0	82·0	64·3	17·7
December	..	64·4	57·5	73·7	61·1	77·0	56·0	21·0
YEAR	..	77·7	72·4	84·7	75·2	86·8	70·5	16·3

TABLE II.  
Precipitation and moisture.

RAINFALL, 1905—1924.				MOISTURE.								
	Average annual amount.	Greatest monthly amount.	Average number of rainy days.*	Extent of cloud whole sky = 10.	Pressure of vapour.				Relative humidity of the air.			
					6 a.m.	2 p.m.	10 p.m.	Mean.	6 a.m.	2 p.m.	10 p.m.	Mean.
January ..	0'36	2'09	0'9	2'3	0'422	0'396	0'465	0'433	87	47	83	73
February ..	1'28	7'96	1'9	2'4	0'494	0'419	0'520	0'481	86	44	78	69
March ..	1'57	6'27	2'6	2'6	0'670	0'540	0'699	0'642	87	42	78	68
April ..	1'93	5'95	3'5	3'3	0'815	0'760	0'833	0'808	87	49	79	71
May ..	5'02	10'01	6'5	4'4	0'890	0'907	0'887	0'899	87	57	80	74
June ..	12'87	31'15	13'5	7'5	0'947	0'990	0'949	0'966	89	71	86	81
July ..	13'97	24'84	15'9	8'6	0'949	0'999	0'966	0'974	91	77	88	85
August ..	13'64	23'32	18'5	8'5	0'945	0'990	0'965	0'969	92	79	89	86
September	9'10	21'42	13'2	7'3	0'930	0'949	0'951	0'949	92	76	89	86
October ..	4'73	14'62	6'0	4'2	0'826	0'785	0'844	0'825	92	66	89	82
November	0'46	3'34	1'2	2'4	0'577	0'529	0'615	0'582	89	52	86	76
December	0'13	1'20	0'5	1'7	0'407	0'379	0'454	0'422	86	46	84	72
Year ..	65'06	86'34	84'2	4'6	0'739	0'720	0'762	0'746	89	59	84	77

\* A rainy day is a day on which 0'10 inch or more of rain is recorded.



TABLE III.

*Wind.*

## NORMALS OF WINDS.

	DIRECTION.			VELOCITY.			
	7 to 8 a.m.	3 to 4 p.m.	10 to 11 p.m.	7 to 8 a.m.	3 to 4 p.m.	10 to 11 p.m.	Mean velocity (m.p.h.).
				Miles per hour.			
January ..	N.16°W.	N.35°W.	N.33°W.	1·5	4·2	1·1	2·2
February ..	N.41°W.	N.71°W.	N.88°W.	1·9	4·7	1·8	2·8
March ..	S.56°W.	S.70°W.	S.33°W.	2·8	5·4	3·1	3·6
April ..	S.25°W.	S.26°W.	S.10°W.	4·6	6·3	4·5	4·9
May ..	S.	S.8°W.	S.5°E.	4·7	6·3	4·4	4·8
June ..	S.6°E.	S.2°E.	S.5°E.	4·4	5·6	3·4	4·3
July ..	S.8°W.	S.3°W.	S.5°W.	4·2	5·0	3·0	4·0
August ..	S.3°E.	S.3°E.	S.3°W.	3·7	4·5	2·5	3·4
September	S.1°W.	S.1°E.	S.1°W.	3·1	4·0	1·9	2·8
October ..	N.28°W.	N.32°W.	N.61°W.	2·2	3·6	1·2	2·1
November	N.2°W.	N.12°W.	N.2°W.	1·9	3·7	0·9	2·1
December	N.8°W.	N.17°W.	N.10°W.	1·7	4·1	0·9	2·1
YEAR ..	..	..	..	3·1	4·8	2·4	3·3

TABLE IV.

*Rainfall.*

Five-day period.	Five-day sums.				1931
	1927	1928	1929	1930	
1	Nil	Nil	Nil	Nil	0·06
2	0·02	Nil	Nil	Nil	Nil
3	0·08	Nil	Nil	Nil	Nil
4	0·27	Nil	Nil	0·58	Nil

TABLE IV—*contd.*

Five-day period.	Five-day sums.				
	1927	1928	1929	1930	1931
5	Nil	Nil	1'15	Nil	Nil
6	Nil	Nil	Nil	Nil	Nil
7	Nil	0'17	0'46	Nil	Nil
8	0'10	Nil	Nil	0'03	Nil
9	1'05	Nil	0'19	0'74	Nil
10	0'03	Nil	Nil	Nil	1'43
11	0'01	0'03	Nil	Nil	1'34
12	Nil	Nil	Nil	Nil	0'13
13	Nil	Nil	Nil	Nil	0'01
14	Nil	Nil	0'15	Nil	1'20
15	Nil	Nil	2'44	Nil	0'10
16	Nil	Nil	Nil	0'29	Nil
17	0'01	0'13	Nil	Nil	Nil
18	0'15	Nil	Nil	0'10	Nil
19	Nil	Nil	Nil	0'04	Nil
20	Nil	1'82	Nil	0'09	1'08
21	0'87	0'50	Nil	0'02	Nil
22	Nil	0'88	0'59	Nil	Nil
23	1'02	Nil	1'46	Nil	Nil
24	0'18	Nil	Nil	Nil	Nil
25	1'55	0'33	0'05	0'19	Nil
26	0'05	4'70	Nil	0'54	0'08
27	2'23	1'11	0'02	2'62	2'26
28	Nil	0'50	0'35	0'03	0'99
29	0'01	0'41	0'51	0'64	1'41
30	1'07	0'11	0'39	0'46	2'70
31	0'75	0'60	0'61	0'58	0'24

TABLE IV—*contd.*

Five-day period.	Five-day sums.				
	1927	1928	1929	1930	1931
32	4'88	0'57	2'74	1'17	Nil
33	0'04	5'21	1'10	0'65	Nil
34	3'58	6'80	0'12	0'64	3'71
35	0'23	1'97	0'43	1'86	0'86
36	1'96	0'74	1'14	3'28	1'45
37	0'07	9'19	0'82	2'62	3'99
38	1'06	3'45	2'34	6'18	4'39
39	3'25	2'01	1'95	2'96	2'25
40	0'34	1'64	0'95	5'53	1'50
41	0'63	4'03	2'06	2'28	1'00
42	2'23	3'84	2'67	1'00	0'03
43	1'98	6'53	1'47	0'29	0'56
44	1'77	1'05	0'50	3'02	0'79
45	1'79	0'59	5'28	0'37	2'00
46	0'49	0'32	1'50	1'69	0'48
47	0'51	4'03	0'83	1'36	1'92
48	0'78	1'20	1'83	2'23	2'92
49	0'62	3'96	1'13	5'85	0'74
50	0'64	0'75	1'70	1'95	4'94
51	4'35	5'69	0'15	1'07	2'31
52	1'28	0'01	1'33	1'68	1'42
53	0'12	0'05	1'36	0'21	0'70
54	0'40	0'14	2'75	1'60	2'99
55	0'27	0'78	3'32	0'51	0'20
56	2'80	0'99	4'56	0'21	1'27
57	Nil	0'53	0'64	0'56	0'12
58	0'10	1'07	0'30	0'29	1'10

TABLE IV—concl'd.

Five-day period.	Five-day sums.				
	1927	1928	1929	1930	1931
59	Nil	0·24	6·00	0·02	0·48
60	Nil	0·29	0·01	Nil	3·10
61	Nil	Nil	Nil	Nil	0·08
62	0·01	Nil	Nil	0·48	2·43
63	0·22	Nil	Nil	0·03	2·61
64	Nil	Nil	Nil	Nil	Nil
65	Nil	Nil	Nil	1·91	Nil
66	Nil	Nil	Nil	0·01	Nil
67	Nil	Nil	Nil	Nil	Nil
68	Nil	Nil	Nil	Nil	Nil
69	Nil	Nil	Nil	Nil	Nil
70	Nil	Nil	Nil	Nil	Nil
71	Nil	Nil	0·09	Nil	Nil
72	Nil	Nil	0·04	Nil	0·01
73	Nil	Nil	0·16	Nil	Nil

TABLE V.

*Number of days in each month in which Rainfall was 0·10 inch or more.*

Month.			1927	1928	1929	1930	1931
January	..	..	1	1	2	1	Nil
February	..	..	2	1	2	1	5
March	..	..	1	1	2	2	3
April	..	..	3	5	2	Nil	1
May	..	..	6	7	4	8	9
June	..	..	11	19	10	13	9
July	..	..	13	21	24	21	16
August	..	..	15	18	18	19	19
September	..	..	12	5	10	15	15
October	..	..	4	6	10	3	10
November	..	..	1	Nil	Nil	3	3
December	..	..	Nil	Nil	1	Nil	Nil

TABLE VI.  
Monthly means of maximum and minimum temperatures and humidity.

Month.	1927.				1928.				1929.				1930.				1931.			
	Monthly maximum temperature.	Monthly minimum temperature.	Monthly humidity. mean		Monthly maximum temperature.	Monthly minimum temperature.	Monthly humidity. mean		Monthly maximum temperature.	Monthly minimum temperature.	Monthly humidity. mean		Monthly maximum temperature.	Monthly minimum temperature.	Monthly humidity. mean		Monthly maximum temperature.	Monthly minimum temperature.	Monthly humidity. mean	
January ..	77.6	58.5	71	%	79.4	58.2	65	%	79.1	59.9	67	%	77.6	55.4	66	%	81.7	59.8	64	%
February ..	82.3	62.0	63		85.9	62.2	57		83.1	60.3	59		83.5	61.6	62		83.3	63.4	68	
March ..	91.2	67.4	58		96.1	70.7	54		92.6	70.2	64		93.2	71.0	61		91.9	73.7	63	
April ..	97.3	77.3	70		96.0	75.7	66		95.9	77.5	72		98.1	72.2	63		97.2	78.5	73	
May ..	94.9	77.6	74		94.5	78.4	76		97.4	81.2	72		95.3	78.9	74		94.4	78.8	76	
June ..	92.0	80.2	84		89.6	78.6	86		92.8	79.4	79		93.3	79.5	81		95.2	81.5	81	
July ..	89.1	79.5	86		88.8	79.0	87		88.0	78.8	87		88.8	78.9	87		89.1	79.3	88	
August ..	88.9	79.0	87		89.5	79.0	86		88.9	78.8	87		88.3	79.1	88		89.9	80.3	88	
September	90.0	78.6	85		91.0	79.5	84		89.7	78.5	84		89.7	79.3	85		89.6	79.4	88	
October ..	89.7	75.3	76		88.5	77.0	84		87.1	74.8	81		89.7	76.5	77		87.0	76.3	87	
November	83.7	65.1	68		84.7	66.5	66		83.4	65.0	69		81.5	66.1	74		81.8	65.8	77	
December	79.0	57.8	69		79.5	58.6	67		76.7	57.4	70		77.7	55.8	67		78.1	59.2	71	

## APPENDIX B.

### STATISTICS REGARDING MALARIA IN CALCUTTA.

TABLE I.

*Cases of malaria and of pyrexia of uncertain origin treated at the Medical Out-patients' Department, Medical College Hospital, 1920-1931.*

Year.	Cases of malaria.	Cases of P. U. O.	Total (Malaria and P. U. O.)
1920	£,803	1,452	7,255
* 1921	..	..	..
1922	2,338	1,183	3,521
1923	2,303	1,057	3,360
1924	2,937	702	3,639
1925	2,393	806	3,199
1926	2,448	693	3,181
* 1927	..	..	..
1928	4,483	383	4,866
1929	4,518	993	5,511
1930	2,550	1,509	4,059
1931	2,880	787	3,667

\* The figures for these years are not available.

TABLE II.

*Cases of malaria treated at the Presidency General Hospital, 1921-1931.*

#### IN-PATIENTS.

Year.	Number admitted.	Number in which parasites were found.	Out-patients treated
1921	454	120	54
1922	342	167	38
1923	228	128	12
1924	227	204	14
1925	230	136	34
1926	161	104	18
1927	163	88	11
1928	195	150	21
1929	166	85	47
1930	168	104	26
1931	173	103	61

TABLE III.

*Cases of malaria treated at the Campbell Hospital, 1929-1931.*

Month.	1929.			1930.			1931.		
	In-door	Out-door	Total	In-door	Out-door	Total	In-door	Out-door	Total
January ..	13	94	107	7	112	119	15	101	116
February ..	8	69	77	10	98	108	9	79	88
March ..	6	63	69	11	80	91	11	110	121
April ..	10	61	71	14	92	106	25	79	104
May ..	13	54	67	9	50	59	21	138	159
June ..	16	52	68	10	71	81	25	161	186
July ..	13	72	85	16	68	84	15	200	215
August ..	15	116	131	19	55	74	17	211	228
September	18	123	141	27	58	85	15	235	250
October ..	21	98	119	24	161	185	21	211	232
November	19	117	136	28	154	182	30	241	271
December	15	95	110	31	91	122	11	193	204
TOTAL ..	167	1,014	1,181	206	1,090	1,296	215	1,959	2,174

TABLE IV.

*Cases of malaria treated at the Mayo Hospital, 1929-1931.*

Year.	Number of malaria cases treated.
1929	42
1930	41
1931	80

TABLE V.

*Cases of malaria among the Calcutta Police, treated at the Police Hospital during the years 1926-1931.*

Year.	Cases in which blood was examined.	Number of positive cases.	Strength of Police Force.
1926	915	92	5,535
1927	1,360	144	5,639
1928	1,324	211	5,637
1929	1,268	179	..
1930	1,002	160	5,747
1931	793	150	..

TABLE VI.

*New cases of malaria (microscopically proved) among employees of the Eastern Bengal Railway, 1930-1931.*

Resident and working in				1930	1931
Corporation limits of Calcutta	..			214	261
Ballyganj	..	..	..	1	7
Dhakuria	..	..	..	0	5
Kalighat	..	..		1	3
Majerhat	..	..		1	9
Dum-Dum Cantonment	..			1	3
Dum-Dum Junction	..				5
Ultadanga Road	..				
Kakurgachi Road	..			0	
TOTAL CALCUTTA AND SUBURBS ..				220	297



TABLE VII.

*Cases of malaria among employees of the Bengal-Nagpur Railway treated at the dispensaries at Garden Reach and Shalimar, 1930-1931.*

	1930	1931
Garden Reach (July—December) ..	5,370	4,656
Shalimar (January—December) ..	1,352	1,071

TABLE VIII.

*Cases of malaria among the employees of the Calcutta Tramways Company, Ltd., treated at Nonapooker Dispensary, 1928-1931.*

Year.	Number treated for malaria
1928	64
1929	94
1930	59
1931	83 *

\* Of the number treated in 1931, 47 were resident in Behala. Fifteen were resident in Central Calcutta, but of these 5 were considered to have undoubtedly received their infection while on leave from Calcutta, malaria fever developing within 10 days of their return from endemic places. Records of place of residence are not available for previous years.

TABLE IX.

*Cases of malaria treated in the Calcutta Hospitals and Dispensaries during the years 1928, 1929, 1930 and 1931.*

(Statistics supplied by the Surgeon-General, Bengal.)

No.	Names of institutions.	1928	1929	1930	1931
1	Calcutta Medical College Hospitals ..	5,029	6,096	3,133	3,941
2	Calcutta School of Tropical Medicine and Hygiene.	3,076	2,518	2,313	2,232
3	Presidency General Hospital .. ..	216	213	194	234
4	Campbell Hospital .. ..	2,246	2,102	1,779	2,190
5	S. N. P. Hospital .. ..	840	881	779	382
6	Chitpore Municipal Dispensary .. ..	1,698	1,692	972	1,207
7	Garden Reach Dispensary .. ..	3,075	2,863	2,361	2,648
8	Kidderpore Municipal Dispensary .. ..	2,532	2,296	2,272	*
9	Kalighat Municipal Dispensary .. ..	846	636	718	1,066

\* Figures not available.

TABLE IX—concl'd.

No.	Names of institutions.	1928	1929	1930	1931
10	Chetla Municipal Dispensary .. ..	869	783	702	912
11	Ballyganj Municipal Dispensary .. .	1,488	1,593	673	1,023
12	Taltolla Municipal Dispensary .. ..	2,528	3,412	4,125	4,078
13	Maniktala Municipal Dispensary .. ..	2,027	1,299	1,567	1,492
14	Ultadanga Municipal Dispensary .. ..	955	1,089	1,052	*
15	Central Municipal Laboratory .. ..	232	328	236	*
16	Gowkhana District I Dispensary .. ..	201	541	812	660
17	Do. II do. .. ..	1,325	1,466	1,337	*
18	Do. IV do. .. ..	939	321	432	462
19	Tangra Municipal Dispensary .. ..	859	1,002	2,090	*
20	Tala Pumping Station .. ..	237	358	420	282
21	Mayo Hospital .. ..	1,968	1,980	1,687	1,244
22	Chandni .. ..	2,914	2,784	2,830	3,085
23	Chitpore .. ..	2,582	2,601	2,550	2,942
24	Carmichael Medical College Hospital, Belgachia	1,520	1,748	2,469	3,447
25	Calcutta Medical School Hospital .. ..	2,772	2,335	2,758	2,946
26	Chittaranian Hospital .. ..	1,410	1,542	1,248	1,591
27	Howrah General Hospital .. ..	363	392	479	959
28	Lady Dufferin Victoria Hospital .. ..	182	112	40	57
29	Calcutta Police Hospital .. ..	368	205	170	136
30	Dispensary attached to the Printing and Stationery Department	..	208	105	167
31	Saibhusan Neogy Dispensary .. ..	2,648	2,674	2,701	3,079
32	Rai Bhagwan Das Bahadur Hospital ..	1,722	2,133	2,919	2,991
33	S. V. S. Marwari Hospital .. ..	2,932	3,380	3,794	4,966
34	Bachulal's Dispensary .. ..	699	498	428	481
35	F. B. Railway Head Office Dispensary ..	301	480	622	500
36	E. I. Railway Head Office Dispensary ..	1,309	234	361	357
37	Baldeodas Maternity Home .. ..	97	350	*	*

\* Figures not available.

## APPENDIX C.

TABLE I.

*Results of blood and spleen examinations among municipal school children,  
February 1932.*

District and Ward.	Name of school.	BLOOD EXAMINATION		SPLEEN EXAMINATION.		
		Number examined	Percentage with parasites	Number examined.	Number with enlarged spleen.	Splenic index (corrected).
DISTRICT I. Ward 1. Shampukur	7-A, Shib Sankar Mullick Lane (H. B.).	100	0·0	177	7-4	
	44, Canal West Road (H. B.).	..	..	92	2-1	
	20-B, Pasupati Bose Lane (Hindi).	..	..	45	3-1	
	7, Haralal Mitra Lane (H. B.)	..	..	90	5-2	
	TOTAL FOR WARD ..	100	0·0	404	17-8	2·2
Ward 2. Kumartuli	26-A, Ananda Khan Lane (H. B. and G.).	..	..	138	5-4	
	42, Banamali Sarkar Street (H. B.)	74	0·0	74	4-2	
	65-A, Nimtollah Ghat Street (H. B.).	26	0·0	97	5-5	
	TOTAL FOR WARD ..	100	0·0	309	14-11	3·0
Ward 3. Burtolla	8-A & H, Jay Mitter Street (H. B.).	68	0·0	75	1-0	
	12-5, Nilmony Mitter Street (H. B.).	32	0·0	96	4-3	
	10-2-A, Gouribari Lane (H. B.).	..	..	101	5-2	
	TOTAL FOR WARD ..	100	0·0	272	10-5	1·8

*N.B.*—The second figure in column 6 indicates the number of children with enlarged spleen who are considered to have certainly acquired infection from outside Calcutta. These cases are omitted in calculating the corrected splenic index given in column 7.

TABLE I—*contd.*

District and Ward.	Name of school.	BLOOD EXAMINATION		SPLEEN EXAMINATION.		
		Number examined.	Percentage with parasites.	Number examined.	Number with enlarged spleen.	Splenic index (corrected).
Ward 4. Sukea Street	19, Panchanan Ghose Lane (H. B.).	100	1·0	113	5-2	
	57-2-B, Raja Dinendra Street (H. B.).	..	..	46	2-0	
	69, Amherst Row (H. B.)	..	..	106	4-2	
	TOTAL FOR WARD ..	100	1·0	265	11-4	2·7
Ward 5. Jorabagan	23-1, Tagore Castle Street (Hindi B.).	70	} 3·0	73	2-0	
	18, Shib Thakur Lane (Hindi B.).	26		48	1-0	
	9, Burtolla Street (H. B.).	..		53	3-2	
	29-2-1, Durponarain Tagore Street (H. G.).	..		74	0-0	
	TOTAL FOR WARD ..	96	3·0	248	6-2	1·6
Ward 6. Jorasanko	10, Simla Street (H. B.)	100	0·0	106	5-2	
	10, Simla Street (H. G.)	..	..	89	1-0	
	13, Bhuban Banerjee Lane (H. B.).	..	..	58	1-1	
	TOTAL FOR WARD ..	100	0·0	253	7-3	1·6
Ward 30. Belgachia	26-B, Nilmony Mitter Road (H. B.).	100	3·0	100	5-3	
	21-11, Nilmony Mitter Road (H. G.).	..	..	100	2-2	
	14-A & B, Anath Deb Lane (H. B.).	..	..	83	4-3	
	6, Khelat Babu Lane (H. G.).	..	..	85	1-0	
	TOTAL FOR WARD ..	100	3·0	368	12-8	1·1

N.B.—The second figure in column 6 indicates the number of children with enlarged spleen who are considered to have certainly acquired infection from outside Calcutta. These cases are omitted in calculating the corrected splenic index given in column 7.

TABLE I—*contd.*

District and Ward.	Name of school.	BLOOD EXAMINATION.		SPLEEN EXAMINATION.		
		Number examined.	Percentage with parasites.	Number examined.	Number with enlarged spleen.	Splenic index (corrected).
Ward 31. Satpukur	2, Rani Branch Road (H. B.).	69	} 20	69	5-2	
	Harey Kristo Sett Lane (H. B.).	31		144	4-4	
	103, South Sinthee Road (H. G.).	..		145	5-1	
	TOTAL FOR WARD ..	100	20	358	14-7	..
Ward 32.	26, Gun Foundry Road (Hindi B.).	75	} 30	75	4-3	
	4-1-A, Kasiswar Chatterjee Lane (H. B.).	25		144	10-7	
	4-1-A, Kasiswar Chatterjee Lane (H. G.).	..		84	0-0	
	TOTAL FOR WARD ..	100	30	303	14-10	1.4
District II. Ward 7. Burra Bazar	194, Cross Street (Hindi B.).	23	} 20	23	1-0	
	29, Clive Street (Hindi B.).	33		34	2-2	
	34, Armenian Street (Hindi B.).	39		39	0-0	
	TOTAL FOR WARD ..	95	20	96	3-2	1.0
Ward 8. Colootolah	3-2-3, Gopal Chandra Lane (H. B.).	100	5.0	103	2-1	
	12, Chattawalla Gullee (Hindi B.).	..	..	69	2-1	
	7-1, Gopal Chandra Lane (H. G.).	..	..	48	0-0	
	TOTAL FOR WARD ..	100	5.0	220	4-2	1.0

*N.B.*—The second figure in column 6 indicates the number of children with enlarged spleen who are considered to have certainly acquired infection from outside Calcutta. These cases are omitted in calculating the corrected splenic index given in column 7.

TABLE I—*contd.*

District and Ward.	Name of school.	BLOOD EXAMINATION.		SPLEEN EXAMINATION.		
		Number examined.	Percentage with parasites.	Number examined.	Number with enlarged spleen.	Splenic index (corrected).
Ward 9. Moochipara	16, Noor Mohammad Lane (H. B.).	100	3.0	148	1-0	
	79-A, Pataldanga Street (H. B.).	..	..	106	7-6	
	1, Chatterji Lane (H. B.).	..	..	181	4-3	
	TOTAL FOR WARD ..	100	3.0	435	12-9	0.7
Ward 10. Bow Bazar	33, Wellington Street (H. B.).	100	4.0	130	3-2	
	17-A, Madan Boral Lane (H. B.).	..	..	95	5-3	
	TOTAL FOR WARD ..	100	4.0	225	8-5	1.5
Ward 11. Puddopukur	Sasi Bhusan Dey School (H. B.).	100	2.0	230	12-9	
	Rajrajeswari School (H. G.).	..	..	196	4-3	
	TOTAL FOR WARD ..	100	2.0	396	16-12	1.0
Ward 28. Belliaghata	152-B, Belliaghata Main Road (H. B.).	100	4.0	137	6-3	
	105, Belliaghata Main Road (H. B.).	..	..	64	4-3	
	105, Belliaghata Main Road (H. G.).	..	..	71	2-2	
	94, Talpukur Road (M. B.).	..	..	79	5-0	
	TOTAL FOR WARD ..	100	4.0	351	17-8	2.5

*N.B.*—The second figure in column 6 indicates the number of children with enlarged spleen who are considered to have certainly acquired infection from outside Calcutta. These cases are omitted in calculating the corrected splenic index given in column 7.

TABLE I—*contd.*

District and Ward.	Name of school.	BLOOD EXAMINATION.		SPLEEN EXAMINATION.		
		Number examined.	Percentage with parasites.	Number examined.	Number with enlarged spleen.	Splenic index (corrected).
Ward 29. Maniktala	19-A, Ultadanga Main Road (H. B.).	100	1'0	101	2-0	
	7-B, Ariff Road (H. G.)	..	..	138	1-0	
	235-B, Maniktala Main Road (M. B.).	..	..	110	3-1	
	235-B, Maniktala Main Road (M. G.).	..	..	34	2-0	
	TOTAL FOR WARD ..	100	1'0	383	8-1	1'8
DISTRICT III Ward 13. Fenick Bazar	33-1, S. Banerjee Road (H. B.).	50	7'4	50	2-2	
	31, Wellesley Street (M. B.).	31		31	3-1	
	10, S. Banerjee Road (H. G.).	..		54	1-0	
	TOTAL FOR WARD ..	81	7'4	135	6-3	2'2
Ward 14. Taltolla	8-1, Sarang Lane (H. B.)	51	0'5	51	3-2	
	7, Walioolah Lane (M. B.).	..	..	90	5-2	
	33-1, Ahmuddin Street (M. B.).	49	6'0	65	3-0	
	75, Durga Charan Doctor Road (H. G.).	..	..	128	4-0	
	TOTAL FOR WARD ..	100	4'0	334	15-4	3'3
Ward 15. Collinga	15-1, Bedford Lane (M. B.).	40	15'0	40	2-1	2'5

*N.B.*—The second figure in column 6 indicates the number of children with enlarged spleen who are considered to have certainly acquired infection from outside Calcutta. These cases are omitted in calculating the corrected splenic index given in column 7.

TABLE I—*contd.*

District and Ward.	Name of school.	BLOOD EXAMINATION.		SPLEEN EXAMINATION.		
		Number examined.	Percentage with parasites.	Number examined.	Number with enlarged spleen.	Splenic index (corrected).
Ward 18. Tangra	37, Christopher Road (H. B.).	97	2.0	98	3-1	
	58-3-A, Christopher Road (H. G.).	..	..	30	0-0	
	4, Prabhu Ram Sarkar Lane (H. B.).	..	..	91	1-0	
	35, Topsia Road South (M. B.).	..	..	37	3-1	
	7, Rai Charan Pal Lane (H. B.).	..	..	46	1-0	
	TOTAL FOR WARD ..	97	2.0	302	8-2	2.0
Ward 19. Entally	20, Ananda Palit Road (H. B.).	100	2.0	120	2-1	
	20, Ananda Palit Road (H. G.).	..	..	121	3-3	
	108-D, Chingrihatta Road (H. B.).	..	..	96	4-3	
	TOTAL FOR WARD ..	100	2.0	337	9-7	0.7
Ward 20. Beniapukur	206, Linton Street (H. B.).	71	0.0	72	3-3	
	260, Linton Street (H. G.)	..	..	86	1-0	
	3, Jannagore 2nd Lane (M. B.).	29	6.8	74	0-0	
	8-2, Hatibagan Road (M. B.).	..	..	56	5-2	
	TOTAL FOR WARD ..	100	2.0	288	9-5	1.4
Ward 21. Ballyganj	100-1, Karaya Road (H. B.).	43	8.0	71	6-2	
	1, Shamsul Huda Road (M. B.).	57		63	0-0	
	25, Bright Street (M. B.).	..	..	64	5-1	
	22, Tiljala Masjidbaree Lane (M. B.).	..	..	38	1-0	
	29, Nurullah Doctor Lane (M. B.).	..	..	54	2-0	
	TOTAL FOR WARD ..	100	8.0	290	14-3	3.8

N.B.—The second figure in column 6 indicates the number of children with enlarged spleen who are considered to have certainly acquired infection from outside Calcutta. These cases are omitted in calculating the corrected splenic index given in column 7.



TABLE I—*contd.*

District and Ward.	Name of school.	BLOOD EXAMINATION.		SPLEEN EXAMINATION.		
		Number examined.	Percentage with parasites.	Number examined.	Number with enlarged spleen.	Splenic index (corrected).
District IV. Ward 22. Bhowanipore	73, Bakul Bagan Road (H. B.).	51	20	52	0-0	
	73, Bakul Bagan Road (H. G.).	..	..	49	1-1	
	72, Harish Mukerjee Road (H. B.).	9	00	9	1-1	
	60-2, Kalighat Road (H. B.).	40	00	93	7-4	
	60-2, Kalighat Road (H. G.).	..	..	76	4-3	
	TOTAL FOR WARD ..	100	10	279	13-9	15
Ward 23. Alipore	7-1, Mayerpore Road (H. B.).	100	20	146	7-6	
	22-A, Pitambar Ghatak Lane (H. G.).	..	..	63	1-1	
	40-B, Judge's Court Road (M. B.).	..	..	33	3-2	
	29-7, Chetla Central Road (H. B.).	..	..	74	2-1	
	TOTAL FOR WARD ..	100	20	316	13-10	10
Ward 24. Ekbalpore	10, Mominpore Road (H. B.).	100	00	114	6-1	
	10, Mominpore Road (H. G.).	..	..	56	2-0	
	7, Mansatala Lane (H. B.).	..	..	61	1-0	
	19-1, Mangstala Lane (Hindi B.).	..	..	105	4-2	
	TOTAL FOR WARD ..	100	00	336	13-3	30

*N.B.*—The second figure in column 6 indicates the number of children with enlarged spleen who are considered to have certainly acquired infection from outside Calcutta. These cases are omitted in calculating the corrected splenic index given in column 7.

TABLE I—concl'd.

District and Ward.	Name of school.	BLOOD EXAMINATION.		SPLEEN EXAMINATION.		
		Number examined	Percentage with parasites.	Number examined.	Number with enlarged spleen.	Splenic index (corrected).
Ward 25. Watganj and Hastings.	12, Mohan Chand Road (M. B.).	88	0.0	89	1-0	
	27-3, Ram Kamal Street (H. B.).	12	0.0	111	6-5	
	10, Bishu Babu Lane (H. G.).	..	..	79	1-0	
	TOTAL FOR WARD ..	100	0.0	279	8-5	1.0
Ward 26. Garden Reach.	96, Paharpur Road (H. B.).	69	} 1.0	69	1-0	
	Burtolla (M. B.) ..	31		126	3-1	
	Dhobapara (M. B.) ..	..		91	5-0	
	TOTAL FOR WARD ..	100	1.0	286	9-1	2.8
Ward 27. Tollyganj	13-1, Nepal Bhattacharjee Street (H. B.).	100	7.0	192	5-5	
	6-A, Nepal Bhattacharjee 2nd Lane (H. G.).	..	..	117	0-0	
	TOTAL FOR WARD ..	100	7.0	309	5-5	0.0

*N.B.*—The second figure in column 6 indicates the number of children with enlarged spleen who are considered to have certainly acquired infection from outside Calcutta. These cases are omitted in calculating the corrected splenic index given in column 7.

TABLE II.

*Results of blood and spleen examinations among children of railway employees, etc., February 1932.*

Locality.	Source of children.	BLOOD EXAMINATION.		SPLEEN EXAMINATION.		
		Number examined.	Percentage with parasites.	Number examined.	Number with enlarged spleen.	Splenic index (corrected)
Kidderpore (Ward 25)	Hooghly Mill employees	100	3·0	103	5-1	
	B. N. R. employees ..	100	3·0	113	2-2	
	TOTAL ..	200	3·0	216	..	2·0
Narculdanga (Ward 19)	E. B. R. employees ..	77	0·0	78	1-0	1·3
Chitpore (Ward 32)	E. B. R. employees ..	88	1·1	90	12-8	4·4
Howrah	Shalimar Rope Works	8	3·3	10	1-0	
	B. N. R. employees ..	112		134	4-1	
	TOTAL ..	120	3·3	144	5-1	2·8

*N.B.*—The second figure in column 6 indicates the number of children with enlarged spleen who are considered to have certainly acquired infection from outside Calcutta. These cases are omitted in calculating the corrected splenic index given in column 7

**APPENDIX D.**  
**SPECIMENS OF FORMS USED IN ANTI-MOSQUITO WORK.**  
*Daily summary of Anti-Malaria Work.*

Ward.....	Inspector.....	Date.....	TOTAL.			
Number of houses inspected	..	..	..	..	..	..
Number of houses found breeding	..	..	..	..	..	..
Number of Containers inspected	..	..	..	..	..	..
Number of Containers breeding	..	..	..	..	..	..
<i>Number of Containers found.</i>						
<i>Description of Containers.</i>						
Wells	..	..	..	..	..	..
Cisterns	..	..	..	..	..	..
Ma-onry tanks	..	..	..	..	..	..
Fountains	..	..	..	..	..	..
Drains (S W E gully traps)	..	..	..	..	..	..
Serviceable articles as tubs, buckets etc	..	..	..	..	..	..
Unserviceable articles as earthenware jars, tins, etc	..	..	..	..	..	..
Root gutters	..	..	..	..	..	..
Others	..	..	..	..	..	..
TOTAL		..	..	..	..	..
<i>Percentage of houses breeding</i>						
Percentage of Containers breeding	..	..	..	..	..	..
<i>Number of breeding-places of Anopheles</i>						
..	..	..	..	..	..	..

## MALARIA.

Monthly report of the Anti-Malaria Work done during the month of ..... 193..... Ward.

	Wells.	Cisterns.	Masonry tanks.	Fountains.	Gully traps and S. W. Eas.	Tubs and receptacles.	Unserviceable articles.	Roof gutters.	Tanks.	Pools and others.
Inspected .. ..										
Breeding .. ..										
Not mosquito-proof .. ..										
Rendered mosquito-proof .. ..										
Treated with larvicide .. ..										
Filled in .. ..										
Hermetically covered or covered with trapdoor. .. ..										
Notices issued under Section .. ..										
Complied with .. ..										
Outstanding .. ..										
Police Court prosecutions instituted .. ..										
Complied with .. ..										
Withdrawn .. ..										
Acquittals .. ..										
Convictions .. ..										
Outstanding .. ..										
Water pumped out or removed .. ..										

Number 0

Inspectors.....  
Sub-Inspectors.....  
TOTAL.

Number 0 children examined for enlarged spleen.....  
Number 0 children with enlarged spleen.....  
Number 0 slides examined for malaria parasites.....  
Number 0 slides in which malaria parasites found.....  
Number 0 complaints for mosquito nuisance received.....  
Number 0 old lids of cisterns replaced by hinged iron mosquito-proof tight fitting ones.....

Malaria Inspector, ..... Ward.

.. MUNICIPALITY.

.193

From

The Assistant Health Officer, Malaria.

To

The Owner or Occupier,

Premises No.....

SIR,

I have the honour to inform you that *Anopheles* mosquitoes were found breeding on the.....on your premises in :—

- |                    |                          |
|--------------------|--------------------------|
| (1) Cisterns.      | (5) Tank.                |
| (2) Well.          | (6) Tubs in the gardens. |
| (3) Fountain.      | (7) Roof gutters.        |
| (4) Masonry tanks. | (8) Odd receptacles.     |

I have the honour to be,

Sir,

Your most obedient servant,

*Assistant Health Officer, Malaria.*

..... MUNICIPALITY.

.....193

From

The Assistant Health Officer, Malaria.

To

.....\.....

*Re* : Provision of access for the cistern.....

at.....

Sir,  
Gentlemen,

I have the honour to inform you that as the storage tanks at your above-mentioned property is/are not accessible and as it is necessary that easy, safe and permanent means of access thereto should be provided so as to facilitate the inspection thereof by the staff of this Department, I have to request you to carry out the following requisition within a fortnight from the date of the receipt hereof, failing which action will be taken against you as provided under Section.....of the Municipal Act.

*Requisition :—*

I have the honour to be,

Sir,  
Gentlemen,

Your most obedient servant,

*Assistant Health Officer, Malaria.*

Notice under Section....of the City of.....Municipal Act.

WARD.

No..... of 193 .

To

Owner of Premises No.....at

.....Street.

WHEREAS in my opinion the well in your above-mentioned premises is or is likely to become a breeding-place of mosquitoes, NOW, pursuant to the provisions of sub-section (1) of Section....of the City of.....Municipal Act, as amended as aforesaid, I do hereby require you within fifteen days from the service hereof to.....'

If you fail to comply with the above requisition, you will render yourself liable to the penalty prescribed in that behalf by Section....of the said Act unless, within the time prescribed for compliance, as aforesaid, you deliver to me written objections to such requisition.

Dated this.....day of.....193 .

*Deputy Executive Health Officer.*



After Conviction Notice (Section.....of the City of.....Municipal Act).

Ward.

No.....of 193 .

To

Owner of Premises No.....at

.....Street.

WHEREAS by Notice No....., dated the.....193 ,  
you were required, pursuant to the provisions of Section.....of  
the City of.....Municipal Act, to.....

And whereas having failed to comply with such requisition within the time prescribed in that behalf by the said notice, you were on the.....193 , convicted and fined for such non-compliance; and whereas it appears to me that the requisitions of the said notice not having yet been complied with, the work hereby required to be done is necessary and must now be undertaken without any further delay.

NOW, I do hereby require you within.....days from the service hereof forthwith to commence to.....

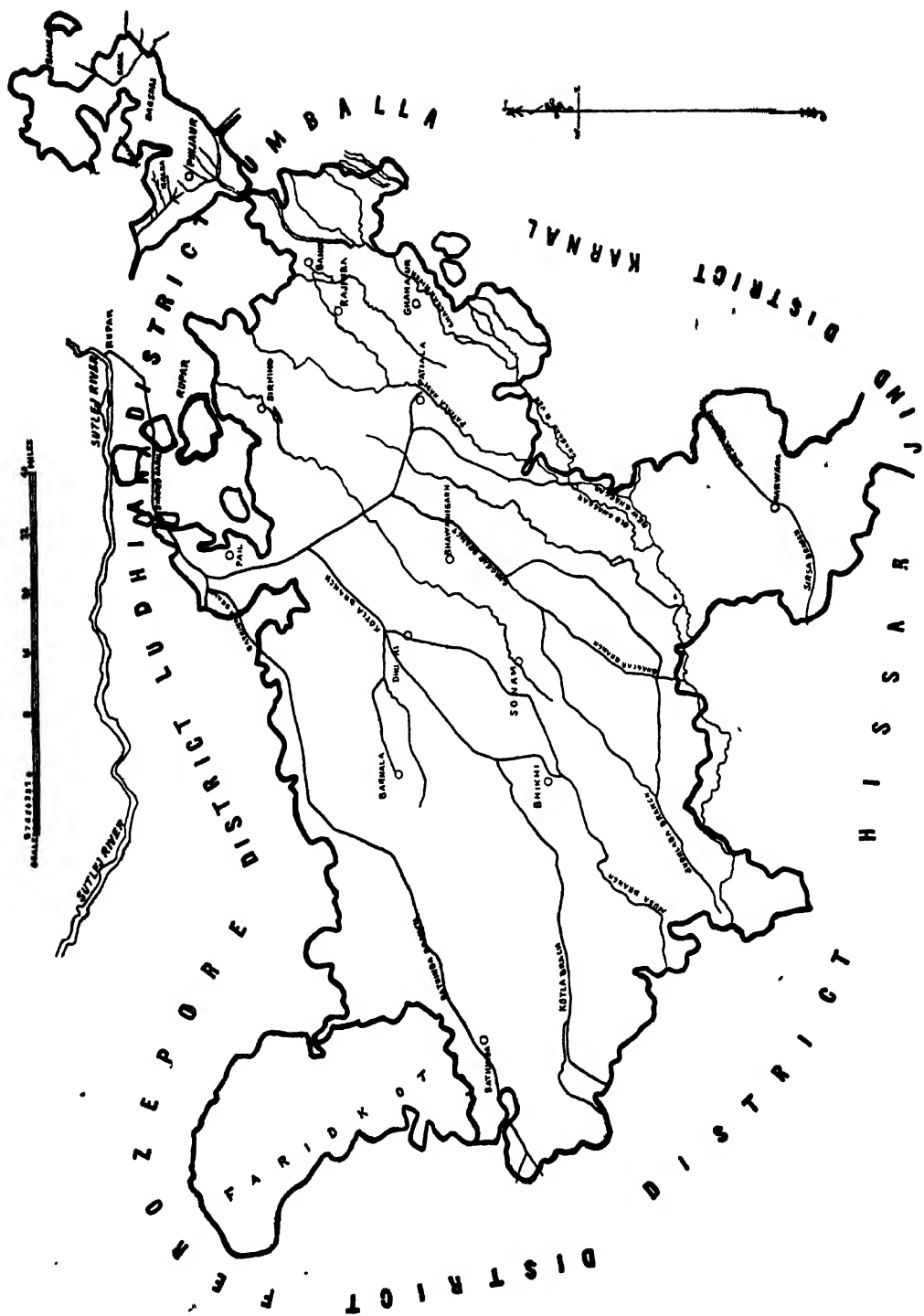
and to diligently carry on the work and complete the same within.....  
.....from the service hereof, failing which you will again be summoned in view to the infliction under Section...of the said Act, of a daily penalty for each day that you continue so to offend.

Take notice also that the requisition herein contained to do the work aforesaid will remain in force until the same be completed and that for all such time as you continue to neglect to carry on and complete the said work you will continue to render yourself liable to such daily fine in respect of such continued offence as is prescribed by Section.....of the said Act.

Dated this.....day of.....193 .

*Deputy Executive Health Officer.*





## MALARIA IN PATIALA STATE.

BY

MAJOR G. COVELL, M.D., D.P.H., I.M.S.,  
*Assistant Director, Malaria Survey of India.*

[January 11, 1931].

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### INTRODUCTION.

THE three Indian States of Patiala, Jind and Nabha in the Punjab are collectively known as the Phulkian States. The main area of this group of States lies between 74° and 77° E. and 29° and 31° N. It is bounded on the north by the District of Ludhiana, on the east by Ambala and Karnal, on the south by Rohtak and Hissar, and on the west by Ferozepore District and Faridkot State. The three States, as a group, hold a comparatively continuous area, though the territory of each is scattered and inextricably mingled with that of its sister States. Several islands of British territory lie within this area, and on the other hand the States possess many outlying villages in British

territory, whilst Patiala holds a considerable area in the Simla Hills. In addition to these possessions, the three States hold a fairly compact block of outlying territory in the south-east of the Punjab, between  $75^{\circ}$  and  $76^{\circ}$  E. and  $27^{\circ}$  and  $28^{\circ}$  N. This block is bounded on the north by Hissar, on the east by Rohtak and Gurgaon, and on the south and west by Rajputana. Each of the States received a part of this territory as a reward for its services in the Mutiny.

Patiala State, with a total area of 5,412 square miles, is considerably the largest and most wealthy of the Indian States in the Eastern Punjab. It is divided into three blocks, as follows :—

(1) The main block forms roughly a parallelogram 139 miles from east to west and 125 miles from north to south, with an outlying tract to the south of the Ghaggar River, which forms part of the *nizamat* of Karnagarh.

(2) The second block lies within the Simla Hills, between  $30^{\circ} 40'$  and  $31^{\circ} 10'$  N. lat. and  $76^{\circ} 49'$  and  $77^{\circ} 19'$  E. long., and is thus comprised within the Himalayan area, where it comes into contact with several of the Simla Hill States, while it is separated on the south from Ambala District by the watershed of the Siwalik Range. This block has a maximum length of 36 miles from north to south, and a breadth of 29 miles from east to west. It forms part of the *nizamat* of Pinjaur.

(3) The third block is the *ilqa* of Narnaul (Mohindargarh *nizamat*), which is remote from the main territory of the State, lying 180 miles to the south of its capital.

The first and second blocks are shown in Map I.

#### *Rivers.*

Patiala State as a whole is badly watered. No great river runs through it or near its borders, and the chief stream which traverses the State is the Ghaggar, which runs from the north-east of the main block in a south-westerly direction. Its bed is narrow and ill-defined in Rajpura and Banur, but in Ghanaur the banks are low and the stream floods easily. Lower down it narrows in places, but generally speaking it is not confined in the rains to any clear or well-defined channel.

#### *Climate.*

The hills, with the exception of the Pinjaur *thana*, have an excellent climate. In Pinjaur, the hot weather is moderate, but the rains are oppressive. In the plains the most healthy parts of the State are the Bangar and Jangal tracts, and the Mohindargarh *nizamat*. The Jangal tract and Mohindargarh have a long and dry hot weather. The mean temperature in Patiala City is usually between  $80^{\circ}\text{F.}$  and  $90^{\circ}\text{F.}$  from April to September, and below  $60^{\circ}\text{F.}$  from the middle of November to the middle of March. Readings as high as

115°F. are sometimes recorded in June and July, whilst the thermometer may fall below 40°F. in December and January. According to the Punjab States Gazetteer, the healthiness of the climate in the various tracts varies inversely with the amount of the irrigation.

### Rainfall.

The rainfall, like the temperature, varies considerably in different parts of the State. In the hills near Simla the average annual fall is between 60 and 70 inches. About Pinjaur and Kalka, at the foot of the Simla Hills, it is about 40 inches, and it decreases as the distance from the Himalayas increases, being about 30 inches at Sirhind, 25 at Patiala and Pail, 20 at Bhawanigarh, and only 12 or 13 at Bhatinda and in the Mohindargarh *nizamat*. In the south-west the rainfall is not only less in amount, but more capricious than in the north and east. In all parts of the State the months of highest rainfall are usually July, August and September. There is generally a small amount in January, known as the 'winter rains', and a slightly larger fall in March. Thunderstorms may occur in April, May and June, but October, November and December are usually completely free from rain (*see* Table I).

TABLE I.

*Monthly rainfall, in inches, recorded in Patiala City, 1921-1931.*

Year.	Apr. 15-May 14.	May 15-June 14.	June 15-July 14.	July 15-Aug. 14.	Aug. 15-Sept. 14.	Sept. 15-Oct. 14.	Oct. 15-Nov. 14.	Nov. 15-Dec. 14.	Dec. 15-Jan. 14.	Jan. 15-Feb. 14.	Feb. 15-Mar. 14.	Mar. 15-Apr. 14.	Total.
1921-22	0'00	0'00	1'10	6'99	1'63	0'56	1'53	0'00	1'28	0'44	0'00	0'04	13'57
1922-23	0'00	0'93	10'62	4'63	0'87	0'54	0'00	0'00	0'00	0'75	0'00	0'00	18'34
1923-24	0'04	0'87	2'57	15'02	3'78	0'20	0'00	1'94	2'90	1'22	0'03	0'00	28'57
1924-25	0'42	0'00	0'43	7'02	9'68	6'36	0'00	1'04	0'00	0'39	0'00	0'97	26'31
1925-26	0'20	0'92	9'08	17'17	0'76	0'00	0'64	0'00	0'77	0'81	1'87	0'86	33'08
1926-27	0'45	1'16	5'54	5'25	5'17	2'03	0'13	0'00	0'00	0'62	3'00	0'00	23'35
1927-28	6'68	1'59	3'63	6'27	3'96	1'58	0'24	0'18	1'51	2'57	0'45	0'36	23'02
1928-29	0'51	1'91	3'97	2'91	4'44	0'00	0'16	2'18	0'00	0'16	0'00	0'91	17'59
1929-30	0'05	0'15	2'42	4'93	2'08	0'00	0'00	0'67	1'15	4'77	0'20	0'40	16'84
1930-31	0'00	0'70	5'65	4'13	1'76	0'00	0'00	0'00	0'98	0'76	3'00	0'00	16'98
1931-32	0'34	0'72	2'82	6'46	5'30	1'25	0'28	0'00	0'54	0'00	0'20	3'30	21'21

*Floods.*

The slope of the country causes floods in some parts of the State in years of heavy rainfall, and these do considerable damage to wells and crops. Patiala, the capital, lies in a depression, and is thus very liable to floods. There were disastrous floods in the years 1887 and 1888, which wrought great havoc. Protective works have since been undertaken to prevent damage to the city, and it is now considered to be secure from the effects of floods.

*Population.*

The population of Patiala State is about 1,500,000. Of these, roughly half are Hindus, the remainder being made up of Sikhs and Muhammadans in about equal numbers.

*Agriculture.*

In the hill tracts potatoes, ginger, turmeric and rice are the most valuable crops, but a good deal of Indian corn is raised. In Pail and Sirhind a fair amount of sugar-cane is cultivated, as also in parts of Patiala, Dhuri and Bhawanigarh. Cotton is grown in all but the sandier tracts, such as Barnala, Bhikki and Bhatinda *tahsils*, and forms the staple produce in Narwana. A certain amount of rice is cultivated in the Sutlej Bet and in Rajpura, Banur and Pinjaur *tahsil*, i.e., in the Ghaggar tract. In Narnaul the chief crop is *bajra*. Wheat is the principal winter crop in the north-western half of the main block of the State, and barley and gram, or a mixture of the two, are the most important winter crops in the south and west. In years of good rainfall there is always a considerable amount of *sarson* (oil-seed) exported from the south and west.

*Irrigation.*

Only 30 per cent of the cultivated area of the State is under irrigation. About two-thirds of this is from canals, and the remainder from wells. The most important canal is the Sirhind Canal, opened in 1882, which takes its origin from the Sutlej at Rupar, and irrigates the greater part of the main block of Patiala State. The Hissar branch of the Western Jumna Canal irrigates a large portion of Narwana *tahsil*. There is also, in the Banur and Rajpura *tahsils*, a small inundation canal which takes off from the right bank of the Ghaggar River about 5 or 6 miles above the old town of Banur, from which it takes its name. In times of heavy flood this used to run as far as Bahadurgarh Fort, but now does not run below the twelfth mile. Little irrigation is done from it in the summer, as in years of ordinary rainfall the country is mostly flooded; while in the cold weather the supply falls so rapidly that the crops are difficult to mature.

*Prevalence of malaria.*

As regards the incidence and severity of malaria in Patiala State, little information of value can be extracted from such statistics as are available. The general opinion expressed by officials and prominent citizens of the State is that the Pinjaur area and the Ghaggar tract generally are intensely malarious; that there is a considerable amount of malaria in Patiala City, though it is very much less than before the installation of the present drainage system, and the erection of the protective bunds; that malaria in the middle portion of the State is moderate in amount; and that in the dry sandy eastern tract, where there is no flooding and irrigation is moderate in amount, malaria is slight. These opinions have in the main been confirmed by the results of the present survey.

The statistics regarding fever cases treated at the Rajinder Hospital, Patiala City, over a number of years (Table II) show that the most malarious months of the year are September, October and November, immediately

TABLE II.

*Fever cases treated at Rajinder Hospital, Patiala City, 1922-1931.*

Month.	1924-1925.	1925-1926.	1926-1927.	1927-1928.	1928-1929.	1929-1930.	1930-1931.	1931-1932.
April 15- May 14. }	253	273	319	495	395	305	218	381
May 15- June 14. }	384	178	344	255	402	294	270	386
June 15- July 14. }	146	197	216	184	348	275	302	283
July 15- Aug. 14. }	166	376	321	374	296	342	333	283
Aug. 15- Sept. 14. }	384	321	524	621	284	600	670	415
Sept. 15- Oct. 14. }	561	385	735	737	275	386	519	565
Oct. 15- Nov. 14. }	403	272	684	524	166	341	450	701
Nov. 15- Dec. 14. }	392	254	386	369	233	273	319	498
Dec. 15- Jan. 14. }	350	244	243	162	192	181	178	284
Jan. 15- Feb. 14. }	224	281	169	95	225	164	175	198
Feb. 15- Mar. 14. }	260	222	152	120	256	187	156	222
Mar. 15- April 14. }	256	286	216	162	324	260	89	166
TOTAL	3,779	3,289	4,309	4,098	3,396	3,608	3,599	4,382



following the close of the monsoon, with a smaller secondary rise in April and May. In 1931, when the monsoon started unusually late, and continued until October, the peak of the malaria season was correspondingly delayed.

#### *Medical and Health Services.*

There are 36 hospitals and dispensaries in the State, seven of these being situated in Patiala City and 29 in districts. The personnel consists of the Director of Medical Services, Civil Surgeon, State Bacteriologist, 15 Assistant Surgeons, 38 male and 7 female Sub-Assistant Surgeons, 78 Compounders, 24 Vaccinators and 2 Inspectors of Vaccination. The total annual expenditure on medical services is approximately 3 15 lakhs of rupees, the income of the State being about 138 lakhs per annum; i.e., the expenditure on medical services represents roughly three per cent of the revenue of the State. This is independent of the amount expended under the head of sanitation.

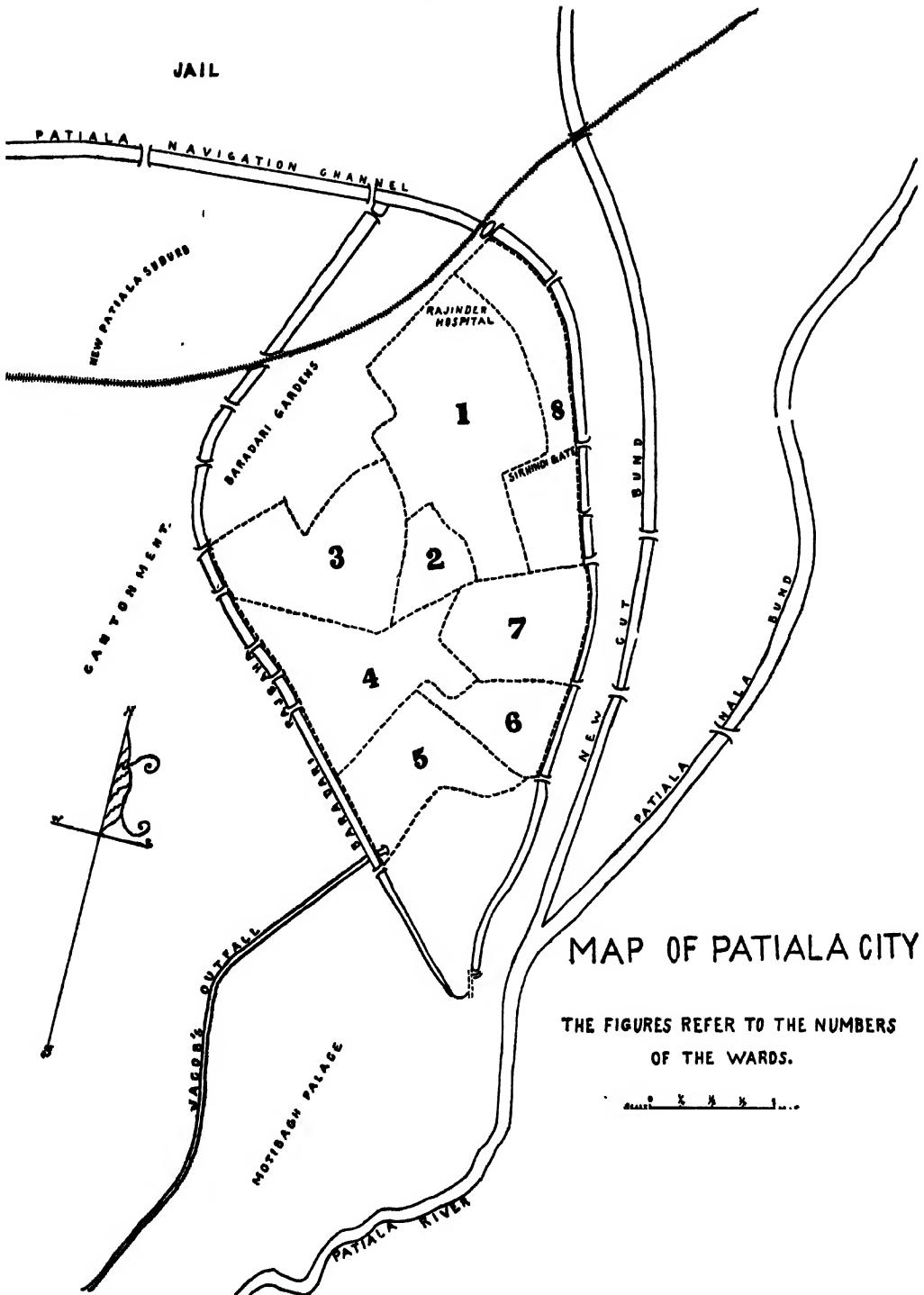
#### **Patiala City.**

PATIALA CITY, the capital of the State, with a population of about 50,000, lies in a depression on the west bank of the Patiala River, on the Rajpura-Bhatinda Railway, 34 miles west of Ambala Cantonment. The houses are mostly built of brick and the main streets are wide and straight, though the lanes leading off from them are narrow and crooked. To the north of the city lie the Baradari Gardens, containing the club, cricket ground, guest house and the residences of various officials. The Rajinder Hospital lies immediately to the south-east of the gardens, beside a large lake called the Rajinder Tank (Map II).

To the east of the city lies a recently constructed residential area called New Patiala, and south of this are the Military Lines, containing well-built barracks. Moti Bagh Palace, the residence of H. H. The Maharaja of Patiala, is situated to the south of the city. There is a large lake in the Palace Gardens, which is supplied from the Baradari Rajbaha.

The Patiala Navigation Channel, the tail of the Patiala branch of the Sirhind Canal, runs along the northern edge of the city, and, turning southwards, skirts also its eastern border. About half-way down the latter, at a point some 400 yards south of the Sirhindi Gate, a bund has been thrown across the canal, and south of this cultivation is carried on in its bed. Above the bund the canal is usually full of water, but has a very slight flow. It irrigates fields and gardens to the south-east of the city and within the city boundary, including part of the Baradari Gardens, and supplies water to the Rajinder Tank. A channel leading off from just above the bund runs along the edge of the canal bed below this, and supplies certain tanks situated at various points in the bed.

# MAP II.





A branch of the Navigation Channel, called the Baradari Rajbaha, runs along the western border of the city, and irrigates the Baradari Gardens, the gardens in New Patiala and the gardens of Moti Bagh. Its tail is carried into the underground portion of the main city drain close to Moti Bagh.

The bed of the Patiala River runs from north to south to the east of the city, lying nearly one mile from it at the north-east corner, but approaching within 400 yards of its south-east corner. From there it curves westwards, running close to the border of the gardens of Moti Bagh Palace. The bed of the river is dry except during the rains. The river finally runs into the Ghaggar.

Formerly the city suffered much from disastrous floods, and there were also a very large number of tanks and depressions within the city boundary. It is now protected by bunds from flooding from the river, and most of the tanks and depressions within the city have been filled in. An excellent system of drainage was installed by Rai Bahadur Ganga Ram, formerly Superintending Engineer, Roads and Buildings, Punjab, about the years 1905 to 1908. In the city itself the drains are brick-lined, and open, with a central cunette. The main drain is finally carried underground, and opens direct into the Patiala River. At the corner of Moti Bagh Palace Gardens there are sluice-gates in the drain, and when the river becomes filled in times of very heavy rain these are closed, and the drainage from the city is diverted into a channel known as Jacob's Outfall, which runs into the river some 16 miles below the city.

To the west of the city there is very little cultivation, a large part of the land being taken up by parade grounds, polo grounds and the aerodrome. On the eastern side however there is much cultivation, chiefly sugar-cane, potatoes, wheat and fruit gardens. There is an extensive area immediately south of the Rajinder Hospital within the city boundary where sugarcane, rice and *arbi* (all wet crops) are grown. Sugar-cane and rice are also grown on low-lying ground between the old bed of the Patiala Navigation Channel and the city walls, south of the bund.

Patiala has a piped water supply from wells, operated by electric power, and chlorinated at the source. This however is not quite sufficient in the hot weather for the needs of the city. There are several hundred wells in the city, but a large number of these are now disused. Some of the wells are actually within the houses. The subsoil water level is about 15 feet.

#### *Amount and distribution of malaria.*

During the month of January 1931, 1,102 children between the ages of two and ten years (chiefly school children) were examined in various parts of the city. Of these, 166, or 15 per cent, showed enlargement of the spleen (see Table II). This indicates that in the city *taken as a whole* the prevalence of malaria is not very great, but a close analysis of the figures obtained reveals the fact that the distribution of the disease varies markedly in different parts

of the city. Of the total number of cases with enlarged spleen, 99, or 60 per cent, lived in Wards I, VII and VIII, and 47 of these were from Ward VIII alone. Out of 37 pupils from a school situated close to the Sirhindi Gate in Ward VIII, 18, or practically 50 per cent, showed splenic enlargement. This is an extremely high rate for a city, and indicates a grave amount of malaria prevalence in this locality.

*Sources of malaria-carrying mosquitoes.*

The eastern wards of the city, which have been shown to be the most malarious, are bordered by the Patiala Navigation Canal. Larvæ of *A. culicifacies*, the chief malaria-carrying mosquito of northern India, have repeatedly been found in this canal, and adults of the same species were found in considerable numbers in cattle-sheds and dwellings in its immediate vicinity in September 1931. There can be no doubt that the severity of malaria in this part of the city is directly due to the numbers of *A. culicifacies* breeding in this canal, and in the seepage water immediately below the bund across its bed.

In Ward I there is a large plot of cultivated land lying immediately to the south-east of the Rajinder Tank and Hospital. In this piece of land (which was, it is understood, originally granted to its present owner on condition that only fruit trees were grown on it) wet crops are raised, i.e., rice, sugar-cane and *arbi*. In September 1931 larvæ of *A. culicifacies* were found in the irrigation water in which the sugar-cane was growing. The plot is irrigated from a supply channel which takes water from the Patiala Navigation Channel to the Rajinder Tank. This supply channel has a brick-lined bed, but it is in a very bad state of repair, and forms a prolific source of breeding for anopheline mosquitoes during the malaria season. There is no doubt that the condition of this channel and the presence of wet cultivation in this area accounts for the high amount of malaria in Ward I, and for the fact that attacks of malaria are so common among patients in the Hospital who have been admitted for other diseases. The presence of wet cultivation along the southern portion of the eastern border of the city probably also accounts in great measure for the considerable amount of malaria in Ward VII.

The cases of malaria which occur in New Patiala can be traced to the breeding of *A. culicifacies* in an unlined irrigation channel which flows through it, and to its distributaries, whilst the Baradari Rajbaha, a channel with grass-grown edges which take off from the Navigation Channel at the extreme north of the city and runs southwards to Mōti Bagh Palace undoubtedly contributes largely to the amount of malaria occurring in its vicinity.

In the Baradari Gardens *A. culicifacies* was found breeding in the basins of fountains, and other ornamental water collections, and the small irrigation channels in this area are also a source of danger.

*Malaria conditions in the Military area.*

There is a considerable incidence of malaria among the troops. In the lines of the 1st and 2nd Infantry and in the Cavalry Lines the chief source of anopheline breeding is supplied by the irrigation overflow channels which run on both sides of the road running through them. A number of adults of *A. culicifacies* were found in the Guard-room which is close to this road. As regards the lines of the 3rd and 4th Infantry the chief source of breeding is a grass-grown irrigation channel originating from the Baradari Rajbaha. Adults of *A. culicifacies* were found in these lines also.

*Malaria conditions in the Central Jail.*

Probably most of the malaria among the prisoners is imported from outside, though certain depressions in the jail compound may become a source of anophelines during the rains. An examination of the children of the warders living outside the jail close by an irrigation channel leading off from the Patiala Navigation Channel showed that about 60 per cent of them had enlarged spleens.

*Recommendations.*

(1) *Anti-malaria staff.*—This consists of one Sub-Assistant Surgeon who has undergone a malaria course at the Field Station of the Malaria Survey of India, Karnal, one Malaria Inspector and 6 coolies. The Sub-Assistant Surgeon, however, has other duties to perform, which make it impossible for him adequately to supervise the anti-malaria work. It is absolutely essential that this officer should be able to devote his *whole time* to anti-malaria work, at any rate for the period July to November. It appears that there are administrative difficulties which make it impossible for him to be spared for whole time work throughout the year; but I learn from the Director of Medical Services that the deputation of this officer for 5 months annually can be arranged without any increase in the present anti-malaria budget of the municipality (Rs. 3,000). I regard the deputation of a whole time officer in charge of anti-malaria operations for at least this five-month period of the year as vital to the success of control measures.

With reference to the remainder of the staff, a second Malaria Inspector is necessary, so that the whole staff will consist of one Sub-Assistant Surgeon, two Inspectors and 6 coolies. For work in the Military area, a squad of 4 men under a N. C. O. should be placed at the disposal of the Sub-Assistant Surgeon in charge of anti-malaria operations in each of the main lines.

(2) *Prohibition of wet cultivation within the city boundary.*—All wet cultivation in this area, including the land lying between the bed of the Patiala Navigation Channel and the edge of the city, should be strictly prohibited.

The Director of Agriculture, Patiala State, has given it as his opinion that the land at present used for these crops is extremely suitable for the growing of fruit trees, and further that the cultivation of the latter will prove far more remunerative than the crops at present being raised.

It may be further pointed out that the crops now grown are not only profusely watered, but are heavily manured with fresh night-soil, and that the sugar-cane crop affords convenient shelter as a latrine, for which purpose it is freely used; so that this type of cultivation is most objectionable within the city limits from the point of view of general sanitation.

(3) The channel conveying water from the Patiala Navigation Channel to the Rajinder Tank should be made 'pukka'. This channel was originally brick-lined so that the work should not be very difficult. If it is re-lined with brick it is essential that it be cement-pointed, in the same way as has been done with the city drains.

(4) The following channels should also be made 'pukka' as funds permit, at the earliest opportunity :—

(a) The irrigation channel running through New Patiala.

(b) The irrigation channel running along the road skirting Moti Bagh, and irrigating the garden of the Palace. This is a grave source of danger to the military guard quartered in this position.

(c) The short channel acting as an overflow for the tail of the Baradari Rajbaha to the covered portion of the city drain. This is in a most neglected state, and as it is only a few yards in length it would be best to put it completely underground.

(d) The channel leading off from the bund across the Patiala Navigation Channel, and running alongside the old disused bed of the canal to supply certain tanks.

(5) A census should be made of all the wells in the city. Those not in use should be oiled, or better provided with cement-concrete covers, as funds permit. Those used for drinking should be similarly covered, but provided with pumps. Trap-doors should not be permitted. Wells are probably of but little importance as regards malaria in Patiala, but they are a major source of the 'mosquito nuisance', and the measures advocated are of importance on the grounds of general sanitation.

(6) The whole course of the Patiala Navigation Channel, where it skirts the city, and also of the Baradari Rajbaha in its whole length, should be treated regularly once weekly with paris green from mid-June to the end of November. Paris green should also be applied regularly to the edges of the Rajinder Tank, Kaulanwala Tank, Moti Bagh Tank, and any others in which the breeding of malaria-carrying anophelines is observed, and also to the small distributary branches of the irrigation channels, and to the fountain basins and other ornamental water in Baradari Gardens.

**Villages in the neighbourhood of Patiala City.**

GHANAUR is a village with a population of 700, situated 17 miles east of Patiala City and 2 miles west of the Ghaggar River. It is said that the prosperity of the village has suffered in recent years owing to three epidemics of plague and the influenza epidemic of 1918, and also to the scarcity of water and bad trade. There are 10 or 12 wells in the village, but water was only present in three of these at the time of the survey (January 1931), the subsoil water level being 35 feet. The chief crops are maize and wheat, and a little cotton is also grown; there is no wet cultivation in the vicinity. The splenic index was 27 (93 observations).

KALYAN is a village with a population of 200, situated 7 miles to the west of Patiala, on the Nabha Road. The Patiala Navigation Channel runs about 50 yards to the south of the village, but does not supply water for the crops in this locality, irrigation being only from wells. The subsoil water level was 20 feet (January 1931). The chief crops are wheat, cotton and sugar-cane. Adults of *A. culicifacies*, *A. subpictus* and *A. fuliginosus* were captured in the village in September 1931. The splenic index (January) was 10 (31 observations).

DADHERA is a village with a population of 100, situated 8 miles to the west of Patiala on the Nabha Road, about 200 yards south of the Patiala Navigation Channel. A small distributary from the latter supplies water to the crops on the western side of the village. Cotton, wheat and sugar-cane are grown. The subsoil water level (January) was 15 feet. The splenic index was 60 (22 observations). In September adults of *A. culicifacies*, *A. subpictus* and *A. fuliginosus* were caught in the village, and the inhabitants stated that malaria was severe.

MADOR is a village with a population of 600, situated 11 miles to the west of Patiala on the Nabha Road, one mile to the north of the Patiala Navigation Channel. The chief crops are wheat, sugar-cane and cotton, and all the irrigation is from wells. The subsoil water level was 19 feet. The splenic index was 3 (78 observations). In September 1931 adults of *A. subpictus* and *A. fuliginosus* only were captured in the village.

RAUNI is a village with a population of 200, situated 5 miles to the west of Patiala on the Nabha Road, about quarter of a mile to the south of the Patiala Navigation Channel. The crops are the same as in the preceding villages, and they are irrigated both from the canal and from wells. The subsoil water level was 20 feet. The splenic index was 17 (24 observations).

KHANSA is a village with a population of 100, situated 5 miles to the south of Patiala on the Bhunarhere Road. The crops are the same as in the preceding villages. The splenic index (January) was 37 (24 observations).

AKANI is a village with a population of 150, situated 6 miles to the south of Patiala, on the Bhunarhere Road. The crops are the same as in the preceding



villages. The subsoil water level (January) was 30 feet, and the splenic index 22 (23 observations).

BAHAL is a village with a population of 200, situated 7 miles to the south of Patiala on the Bhunarhere road. The crops are mainly wheat and cotton, there being no wet cultivation except a little sugar-cane. The subsoil water level (January) was 36 feet, and the splenic index 22 (32 observations).

CHUHARPUR MARASIAN is a village with a population of 200 situated 7 miles to the west of Patiala on the Sunam Road. The chief crops are wheat, barley, sugar-cane and cotton, and irrigation is by wells. The subsoil water level (January) was 18 feet, and the splenic index 35 (31 observations). In September adults of *A. culicifacies*, *A. subpictus*, *A. fuliginosus* and *A. pulcherrimus* were caught in the village, and the inhabitants stated that fever was rife. It is noteworthy that the dwellings in this village contain very large rooms, and that cattle are housed in them as well as human beings. In the daytime straw is burnt in them to drive out the mosquitoes.

BHEDPURA is a village with 250 inhabitants, situated 10 miles to the west of Patiala on the Sunam Road. The crops are the same as at Chuharpur, and there is no canal irrigation. Sugar-cane is grown within 100 yards of the southern border of the village. The subsoil water level (January) was 19 feet, and the splenic index 5 (62 observations). At a subsequent visit in September the villagers stated that there were a number of cases of fever.

SULTANPUR is a village with 100 inhabitants, situated on the Sunam Road 9 miles west of Patiala. Conditions are similar to those in Bhedpura. The subsoil water level (January) was 19 feet, and the splenic index 21 (19 observations). In September the villagers stated that fever cases were numerous.

BHAWANIGARH is a rural town with a population of 3,000, situated 23 miles west of Patiala, on the Sunam Road. The chief crops are maize, cotton, sugar-cane, pulses, wheat, barley and garlic, and irrigation is mainly from wells. The soil is mostly a sandy loam. There is a canal distributary which is chiefly used for filling the Gurdwara Tank, originating from a minor running 2 miles to the east of the town. There are numerous ponds round the circumference of the town. The subsoil water level (January) was 12 feet, and the splenic index 2.5 (284 observations). Adult specimens of *A. culicifacies*, *A. fuliginosus* and *A. pulcherrimus* were caught in September.

PASYANA is a village with 800 inhabitants, situated on the Samana Road, 5 miles south-west of Patiala. The type of cultivation is the same as that in the preceding villages, irrigation being from wells. The splenic index (January) was 23 (95 observations), but a number of the children with enlarged spleens came from a village lying two miles to the east, which has canal irrigation.

DILAWANPUR is a village with a population of 200, situated 8 miles south-west of Patiala on the Samana Road. The village lies about quarter of a mile to the north of a minor canal, but the irrigation in the vicinity is exclusively

from wells, the crops being the same as in the case of the preceding villages. The splenic index in January was 20 (25 observations).

BHANRA is a village with 700 inhabitants, situated  $8\frac{1}{2}$  miles from Patiala on the Samana Road. The general conditions appeared to be much the same as at Dilawanpur, but the splenic index in January was only 6 (94 observations).

BADSHAHPUR is a small village 30 miles south-west of Patiala, close to the bed of the Ghaggar River. The tract in which it lies has the reputation of being intensely malarious. A considerable amount of rice is grown in the vicinity. The writer was unable to visit this village, but the Director of Medical Services, Patiala State, found a splenic index of 90 in October 1931 (20 observations).

Details of the spleen examinations made in the various villages are given in Table III, and the results of catches of anopheline larvæ and adults in Table IV.

TABLE III.  
*Results of spleen examination of children.*

Town or village.	Number examined.	Number with enlarged spleen.	Splenic index.	A-U measurement of average enlarged spleen (cm.).
Patiala City ..	1,102	166	15	9.4
Ghanaur .. ..	93	25	27	9.2
Kalyan .. ..	31	3	10	10.3
Dadhera .. ..	22	13	60	9.7
Mador .. ..	78	2	3	11.5
Rauni .. ..	24	4	17	7.5
Khansa .. ..	24	9	37	8.4
Akani .. ..	23	5	22	9.8
Bahal .. ..	32	7	22	10.6
Chuharpur Marasian ..	31	11	35	9.7
Bhedpura .. ..	62	3	5	11.0
Sultanpur .. ..	19	4	21	9.7
Pasyana .. ..	95	22	23	10.3
Dilawanpur .. ..	25	5	20	10.8

TABLE III—concl'd.

Town or village.	Number examined.	Number with enlarged spleen.	Splenic index.	A-U measurement of average enlarged spleen (cm.).
Bhanra .. ..	94	6	6	11·8
Bhawanigarh ..	284	7	2	10·1
Pinjaur .. ..	57	33	58	9·6
* Bhatinda .. ..	312	19	6	10·6
* † Badshahpur ..	20	18	90	..

\* The observations in Bhatinda and Badshahpur were made in September 1931. The remaining figures were obtained in January 1931.

† The observations in Badshahpur were made by the Director of Medical Services, Patiala State.

TABLE IV.

*Species of anopheline mosquitoes captured, September 1931.*

Town or village.	<i>A. culicifacies.</i>		<i>A. subpictus.</i>		<i>A. fuliginosus.</i>		<i>A. pulcherrimus.</i>	
	Adults.	Larvæ.	Adults.	Larvæ.	Adults.	Larvæ.	Adults.	Larvæ.
Patiala City ..	+	+	+	+	+	+	+	+
Bhawanigarh ..	+	..	+	..	+	..	..	..
Chuharpur Marasian	+	..	+	..	+	..	+	..
Bhatinda ..	..	..	+	..	..	..	..	..
Dadhera ..	+	+	+	..	+	+	..	..
Kalyan ..	+	..	+	..	+	..	..	..
Mador ..	..	..	+	..	+	..	..	..

*Possibility of controlling malaria in villages.*

The control of malaria in rural areas always presents great difficulties, on account of the disproportion between the sum necessary for control and the size and financial value of the villages. From the above account it will be seen that the incidence of the disease varies very considerably in different villages in the neighbourhood of Patiala City, according to local conditions. Malaria control measures throughout the State are out of the question for

financial reasons; but it might be possible for the malaria unit in Patiala City to extend its activities into certain of the villages in the neighbourhood which are situated on good roads, and where much could be done by treating certain breeding-places. For instance, malaria in the villages alongside the Patiala Navigation Channel is almost entirely due to the breeding of numerous anophelines in its grassy edges, and in the small distributary channels. If a motor lorry were provided, it would be possible for a malaria inspector to visit a number of these villages in a single day, and treat the edges of the canal and its distributaries with paris green in the immediate neighbourhood of each village. Similar work might be done in the villages lying on the other roads which radiate from the capital.

As regards malaria in the Ghaggar tract, the only practical means of amelioration in our hands at present is to provide increased facilities for treatment as far as possible.

### **Bhatinda.**

BHATINDA is an important railway centre, situated in the midst of the Jangal tract, 96 miles west of Patiala. The climate is hot and dry, the average annual rainfall being only 8 to 10 inches. There is a large fortress in the town, in which a detachment of troops is quartered, and a visit was paid in September 1931 to investigate malaria conditions and to advise on any control measures which might be thought necessary. The population of the town is 23,000. The chief crops in the vicinity are bajri, oil-seeds, gram and juar, and no wet crops are grown. The sub-soil water level was 120 feet. The Sirhind Canal runs one mile to the north of the town, but the country in the vicinity is only lightly irrigated. The splenic index among school children was 6 (312 observations), but several of the children with enlarged spleens were recent arrivals, and the incidence of malaria in the town is evidently very slight indeed. The only species of anopheline caught was *A. subpictus*. It is not considered that any anti-malaria measures are called for.

### **Pinjaur.**

PINJAUR, the headquarters of the Pinjaur *tahsil*, is situated 3 miles south of Kalka on the Ambala-Simla Road, at the confluence of the Koshallia and Jhajra, two tributaries of the Ghaggar River. The chief crops are rice, cotton, sugar-cane and wheat. The population in 1901 was 800, but it is now not much more than half that number. It is the head-quarters of the Conservator of the Patiala State Forests, and is noted for the beautiful State Gardens which are laid out after the model of the Shalamar Gardens at Lahore. The village has always had an evil reputation for malaria, and is extremely unpopular among officials on that account. The conditions present a typical example of malaria as it occurs in the submontane areas of northern India. There are a

TABLE V.  
Results of adult catches of anopheline mosquitoes in Pinjaur from May 1931 to April 1932.

Malaria in Patiala State.																										
1931												1932														
12. v.	26. v.	9. vi.	23. vi.	7. vii.	21. vii.	4. viii.	18. viii.	1. ix.	15. ix.	29. ix.	13. x.	27. x.	10. xi.	24. xi.	8. xii.	23. xii.	5. i.	19. i.	2. ii.	16. ii.	1. iii.	15. iii.	29. iii.	19. iv.	TOTAL.	
<i>A. listoni</i> ..	38	38	39	17	69	38	44	16	20	34	24	195	100	116	92	93	97	81	129	129	116	141	196	202	234	2,306
<i>A. culicifacies</i> ..	9	17	31	21	53	51	59	77	32	67	26	86	40	19	36	10	9	8	4	2	0	1	2	4	16	680
<i>A. maculatus</i> ..	2.	2	2	1	13	13	12	5	3	7	9	3	7	22	13	6	5	4	14	16	11	9	2	2	2	185
<i>A. stephensi</i> ..	0	0	2	1	2	2	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	9	
<i>A. maculipalpis</i>	18	16	21	15	8	3	9	2	2	2	0	5	1	2	0	4	0	0	0	2	6	3	5	54	178	
<i>A. fuliginosus</i> ..	15	15	3	4	8	13	52	27	92	31	109	3	3	1	2	1	1	0	1	0	0	3	12	14	408	
<i>A. subpictus</i> ..	0	0	2	0	14	71	136	241	98	130	63	45	29	12	1	1	2	0	0	0	0	0	1	1	947	
<i>A. turkhudi</i> ..	0	2	5	4	7	4	11	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	2	37	
<i>A. pallidus</i> ..	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	2	
<i>A. hindustani</i> ..	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	3	1	2	1	1	1	0	1	0	11	
<i>A. gignis</i> ..	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	
<i>A. aithenii</i> * ..	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	
												Total ..													4,668	

\* Note while going to Press.—Subsequent examination has shown that this specimen is not *A. aithenii*, but a species not hitherto described

\* Note while going to Press.—Subsequent examination has shown that this specimen is not *A. aithenii*, but a species not hitherto described.

number of springs, small streams, and seepage outcrops in the vicinity, which form ideal breeding-places for malaria-carrying mosquitoes, and there are also several stone-lined tanks. Two of these are covered in, and water from these is taken by pipes to supply the State Gardens. The splenic index in January was 58 (57 observations).

Routine catches of adult anopheline mosquitoes were made fortnightly in the village from May 1931 to April 1932, and the results are given in Table V. Though individuals of 12 species were captured during this period, only four were found in sufficient numbers to exert any appreciable effect on the transmission of malaria. These were *A. listonii*, *A. culicifacies*, *A. fuliginosus* and *A. subpictus*.

The two last-named species may be safely ignored as malaria carriers in Pinjaur. It will be noted that the catches of both these species, which had been the most abundant since mid-July (soon after the commencement of the monsoon), dropped almost to zero at the end of September, the disappearance of *A. fuliginosus* being particularly sudden. In other words, these two species practically disappeared just before the commencement of the malaria season. Apart from this, the evidence is overwhelming that neither species plays any significant part in malaria transmission in northern India.

The catches of *A. culicifacies*, which species had been fairly prevalent since the beginning of July, decreased markedly towards the end of October. *A. listonii*, on the other hand, showed a sudden and dramatic rise at the beginning of October, i.e., just before the commencement of the malaria season, and remained by far the most abundant anopheline during the next 3 months. This species is the only one which was found infected in Pinjaur during the period under review (see Table VI).

TABLE VI.

*Results of dissections of anopheline mosquitoes caught at Pinjaur.*

Month.			<i>A. listonii.</i>	<i>A. culicifacies.</i>	<i>A. maculatus.</i>
August	Dissected	..	13	32	1
	Infected	..	0	0	0
September	Dissected	..	52	82	12
	Infected	..	2 (gut only)	0	0
October	Dissected	..	155	55	5
	Infected	..	0	0	0
November	Dissected	..	141	49	22
	Infected	..	0	0	0
December	Dissected	..	87	5	12
	Infected	..	0	0	0
TOTAL	Dissected	..	448	223	53
	Infected	..	2	0	0

It is interesting to note that *A. maculatus*, which has in the past been suspected as a carrier in the Himalayan foot-hills, mainly on the strength of its evil reputation in Malaya, was only captured in very scanty numbers in Pinjaur, chiefly in cow-sheds. On all the evidence, it is considered that *A. listonii* is the chief malaria carrier in this area, with *A. culicifacies* probably playing a minor part in transmission.

#### *Recommendations for Pinjaur.*

(1) All the seepage outcrops and streams alluded to above situated within a radius of half a mile from the periphery of the village and State Gardens should be treated regularly once a week with paris green from the middle of June to the end of November.

(2) One or more of the stone-lined tanks should be reserved for drinking purposes. These should be stocked with *Gambusia* fish, which have already been introduced into one of the tanks. The remaining tanks should be treated with paris green as above if it is found that malaria-carrying anophelines breed in them.

(3) In the State Gardens the water supplying the central stone-lined channel, water-falls and fountains should be turned full on at least once a week, preferably twice, with the object of washing out any larvæ which may be present. The small irrigation channels running parallel with the main channel should be lined with stone or brick, and cement-pointed, or lined throughout with cement. The entire system of irrigation and drainage of the Gardens needs thorough overhauling by engineers. It appears that the gardeners have been in the habit of stopping up certain of the underground channels to irrigate special parts of the grounds. This has led to dangerous seepage in several places. Probably if the system were overhauled and the gardeners prevented from interfering with it the seepage would be prevented.

(4) The buildings within the Gardens used for human habitations should be screened with mosquito-proof gauze. This will be an easy task, for the doors and windows are already fitted with wire gauze. The latter is however in a bad state of repair, and moreover the size of mesh of the wire is too large to exclude all dangerous mosquitoes. The type of gauze recommended is 'Rustless Bronze Gauze Screen', 14 mesh and 28 S. W. G.

(5) *Anti-malaria staff.*—One Malaria Inspector and 3 coolies should be engaged, to work under the supervision of the Sub-Assistant Surgeon in charge of the Dispensary at Pinjaur. The officer in charge of anti-malaria operations in Patiala City should also visit Pinjaur regularly to inspect the work.

(6) It might also be possible to administer small doses of plasmochin at weekly intervals to the inhabitants of the village, especially the children, with a view to destroying the gametocytes of malignant tertian malaria, and thus

rendering the population non-infective to anophelines. If this could be done it is essential that the doses should be actually administered by the Sub-Assistant Surgeon in person.

SUMMARY.

(1) An account is given of a malaria survey of certain towns and villages in Patiala State, carried out in 1931.

(2) Malaria in the State is most severe in the Himalayan foot-hills, and in the Ghaggar tract.

(3) *A. culicifacies* is considered to be the principal malaria carrier in the plains, and *A. listonii* in the foot-hills.

(4) Detailed recommendations are made for the control of malaria in Patiala City and the surrounding villages, and in Pinjaur.





## MALARIA IN SIND.

### Part VIII.

#### MALARIA IN SHAHBANDAR DIVISION, KARACHI DISTRICT, SIND

BY

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AND

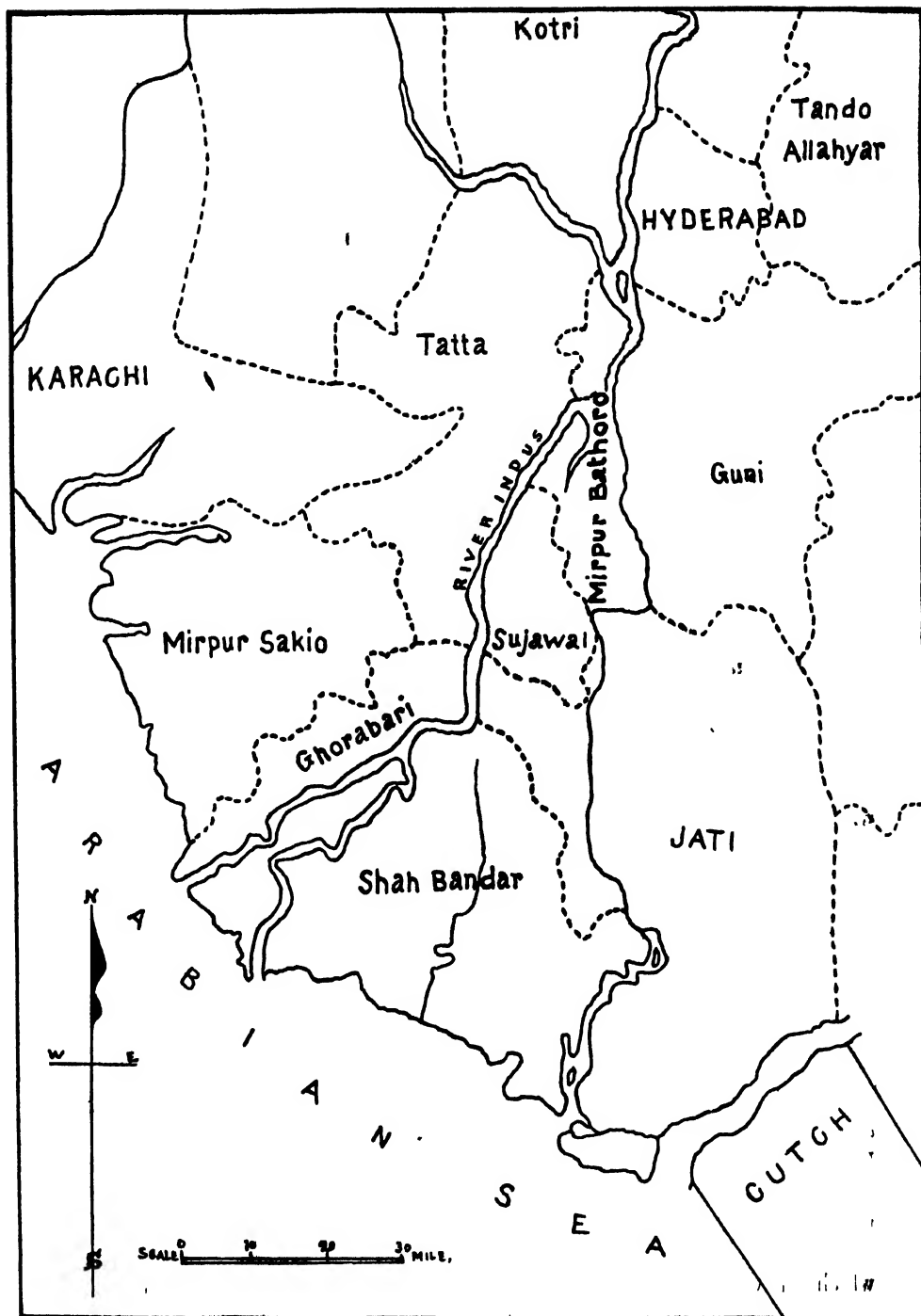
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[January 11, 1932].

AN investigation into the prevalence of malaria in Shahbandar Division was undertaken by the Officer-in-Charge, Sind Malaria Inquiry, in response to a request from the President of the District Local Board, Karachi, through the Government of Bombay to the Government of India. The survey was conducted out from September 12th to 26th, 1931, and investigations were carried out in 55 villages. As far as can be ascertained no previous systematic survey of the division had been previously carried out. The division lies entirely outside the area to be commanded by the Sukkur Barrage Scheme.

#### GENERAL DESCRIPTION.

Shahbandar is the southernmost division of Karachi District, and is composed of the four talukas of Mirpur Bathoro, Sujawal, Jati and Shahbandar (*see Map*). The division covers an area of 4,189 square miles containing 351 rural towns and villages, and consists of a flat low-lying plain, intersected by numerous creeks and channels. The river Indus enters the division at its northernmost point, and running southwards divides into two branches, one of which, the Ochito, runs westwards into the Ghorabari Taluka of Tatta Division,



whilst the other, the Haideri, runs southwards to the sea, forming the western boundary of Shahbandar Taluka. The whole division forms part of the delta of the Indus, but whereas the northern portion is largely fertile rice country the most southern portion is barren, and consists of sandhills, mangrove swamps and salt waste.

The climate is good, since the fierce heat of the summer is tempered by the breeze from the sea, which blows steadily from W-S-W from April to October. While this lasts the thermometer rarely rises above 93°F or falls below 75°F., while the humidity of the air ranges from 75 to 85 per cent. Humidity is very variable during the cold season, but the air is never dry with the dryness of northern India. In November the land breeze sets in, and for four months the prevailing direction is E-N-E, the temperature gradually sinking until it may range for days together between 60°F. and 40°F.

The rainfall is extraordinarily variable from year to year. It is usually very slight in amount, only two or three inches, but sometimes there may be 15 to 20 inches in the year, due to cyclonic disturbances. Most rain falls in August, July and September, but there may be none in these three months. There are normally two periods of rain with two dry intervals, for October and November are practically rainless, and April and May nearly so.

According to the Sind Gazetteer, notwithstanding all that has been accomplished in restraining the annual overflow of the Indus by protective bunds, it is probable that the lowlands of the Delta will never be safe from occasional destructive floods. In the past such floods have been frequent and sometimes disastrous, as in 1892, when heavy rain, combined with floods from the hills, covered the face of the country with water, breaking the canals and almost totally destroying the kharif crops in Shahbandar Division, and causing widespread distress. In 1914, however, when the river gauge reading was the highest on record, the bunds of the Shahbandar Division stood, though that in Ghorabari Taluka burst, with disastrous results.

Irrigation is from the Indus, or from canals taking off from it, but these canals are really old channels of the Indus, formed by the river changing its course. At the present day many of these are provided with regulators. The chief canals are the Pinyari, Gungro, Sattah and Khanto with their branches. In addition there are many small canals which take off directly from the river.

The total cultivable land of the division is about 325,000 acres, of which roughly 200,000 are actually cultivated, 87 per cent of the cultivation being rice. In some low-lying areas, especially in parts of Sujawal Taluka, there are a number of natural depressions covering areas of several square miles, which become filled with water draining from higher areas and from the tail discharges from small canal distributaries. These are locally known as 'dhands'.

Some of these are situated in channels which were formerly beds of the river, but which now serve the purpose of drainage channels. A kind of rice known as 'motia' is sown in the beds of these 'dhands', an embankment being frequently constructed to retain the water. If the rise of water during the inundation season is gradual the rice plants are able to keep pace with it, but should it be sudden, they are overtopped and destroyed. One such 'dhand' called Pinila, covering an area of about 10 square miles, lies six miles north-east of Sujawal in the course of a drainage channel called 'Nagan Dhoru'. This channel after taking its origin from the river near a village called Belo, passes south and finally discharges into the Pinyari Canal about a mile south of Mirzo Laghari village.

#### INCIDENCE OF MALARIA.

A study of the various factors influencing the prevalence of malaria in Lower Sind has shown that there is usually an increased incidence of the disease in years of excessive rainfall combined with a high river level persisting into the months of September and October. This may be accounted for by the increase in atmospheric humidity thereby produced, which is favourable to the longevity of mosquitoes, and by the increased facilities for anopheline breeding provided by the water in the canals and their distributaries remaining for a longer period.

As regards the seasonal incidence of malaria, as far as can be judged from the dispensary records and from local inquiries, the main malaria season in Shahbandar Division is from September to December, the most malarious months being November and October. In certain years there is a minor rise in malaria incidence in March and April.

The average monthly attendance per 10,000 of population at the four taluka dispensaries of the division are given in Table IV.

#### Results of the Survey.

##### 1. MIRPUR BATHORO TALUKA.

This taluka has an area of 269 square miles, and forms the most northern part of the division. It contains 65 villages with a total population of about 42,000. The taluka is a well watered alluvial plain, the northern portion being supplied by canals fed directly from the Indus, and the central and southern parts by distributaries of the Pinyari Mulchand canals. The finest rice, known as *sugdasi*, is grown here. *Jowar* and *bajra* are also grown.

The results of spleen examinations in this taluka are shown in Table I.

TABLE I.  
Mirpur Bathoro Taluka—Results of spleen examinations.

Name of village.	Population.	CHILDREN.				ADULTS.			
		Number examined.	Number with enlarged spleen.	Percentage with enlarged spleen.	A-U measurement of average enlarged spleen (cm.).	Number examined.	Number with enlarged spleen.	Percentage with enlarged spleen.	A-U measurement of average enlarged spleen (cm.).
Mirpur Bathoro .. ..	1,200	105	65	62	7.2	90	37	41	6.0
Fateh Mohamed Shah ..	75	14	6	43	7.7	18	12	67	3.1
Bazi Gar .. ..	300	28	14	50	8.8	22	10	45	5.1
Maman, Achar, and Umar Baroch	120	44	25	57	6.5	30	12	40	4.3
Zangi Zor .. ..	40	12	2	17	..	9	1	11	..
Ahmed Khan Zor and Rahim Khan Zor.	90	15	7	47	11.0	23	3	13	..
Chang .. ..	100	26	10	38	8.3	17	2	12	..
Got Shora .. ..	50	11	5	45	..	5	3	60	..
Joakh .. ..	600	61	24	39	7.7	59	16	25	6.8
Daro .. ..	900	188	69	37	7.8	77	24	31	6.7
Laikpur .. ..	200	47	17	36	7.4	52	17	33	7.1
Bano .. ..	460	42	26	62	8.6	52	23	44	6.0
Sabu Khudai .. ..	60	12	7	58	9.6	16	3	19	..
Sidik Shora .. ..	70	11	10	91	5.4	14	7	50	3.8
Dari .. ..	125	20	13	65	5.7	12	2	17	..
TOTAL .. ..	4,290	636	300	47	7.6	496	172	35	5.7

MIRPUR BATHORO, the headquarters of the taluka, with a population of 1,200, is situated on a slightly elevated site, and is traversed by a minor canal called the Fateh Wah, which passes from north to south through the western wing of the town. To the south of the town there is a tank with an area of

about four-fifths of an acre, supplied by the minor above referred to. The overflow from this tank together with the 'pancho' water drained off from the adjacent ricefields collects in a low-lying area covering some 25 acres beyond the tank in the inundation season. There are two other tanks on the north-west and eastern boundaries of the town, and there are a number of depressions and borrow-pits surrounding the town into which water from the ricefields is drained. The subsoil water level at the time of the survey was 4 feet.

Larvæ of *A. culicifacies*, the chief malaria-carrying mosquito of Sind, were collected along the grassy edges of the tanks, and in many places in the large sheet of water referred to lying to the south of the town. Larvæ of *A. subpictus* and *A. fuliginosus* were obtained from borrow-pits along the edges of the ricefields. In the town adult specimens of *A. culicifacies*, *A. subpictus*, *A. fuliginosus* and *A. pulcherrimus* were caught. The splenic index was 62 (105 observations), and the parasite index 52 (25 observations).

FATEH MOHAMED SHAH is a village with a population of 75, situated one mile south-west of Mirpur Bathoro in the midst of rice cultivation. A small canal distributary runs through the centre of the village, and a 'dhand' covering an area of about 200 square feet lies close to the periphery. There are no wells. Larvæ of *A. culicifacies* were collected along the edge of the distributary, and those of *A. subpictus* from the 'dhand'. The splenic index was 43 (14 observations).

BAZI GAR is a village with a population of 200, situated quarter of a mile south of Mirpur Bathoro, at the edge of the large sheet of water above referred to. The splenic index was 50 (28 observations).

MAMAN, ACHAR and UMAR BAROCH form a group of villages with a total population of 200, situated in the midst of rice cultivation 2 miles south-west of Mirpur Bathoro. A canal distributary runs within a short distance of the villages, and each village has a 'dhand'. In addition there are a number of borrow-pits and excavations surrounding the villages into which water from the ricefields drains. The water in the distributary dries up in October, and that in the 'dhand' remains till December.

Larvæ of *A. culicifacies* were obtained in the distributary, and those of *A. subpictus* in most of the borrow-pits. Adults of both these species were caught in the villages. The splenic index was 57 (44 observations).

ZANGI ZOR is a village with 40 inhabitants, situated 1½ miles south-west of Mirpur Bathoro in the midst of rice cultivation. A minor canal runs about 600 yards from the village. Larvæ of *A. pulcherrimus* were found in collections of water along the edge of ricefields, and those of *A. culicifacies* in the bed of a distributary which was nearly dried up. Adult specimens of *A. subpictus*, *A. culicifacies*, *A. pulcherrimus* and *A. fuliginosus* were caught in the village. The splenic index was 17 (12 observations only).

AHMAD KHAN ZOR and RAHIM KHAN ZOR are two villages with a combined population of about 90, situated in the centre of rice cultivation  $1\frac{1}{2}$  miles south-east of Mirpur Bathoro. A minor canal runs about 600 yards from the villages, and a distributary from this skirts the villages. There is no village 'dhand', but water drained from the ricefields collects in the many excavations which surround the village. Larvæ of *A. culicifacies* and *A. subpictus* were found in this water, and those of *A. pulcherrimus* in collections of water along the edges of the ricefields. The splenic index was 47 (15 observations).

CHANG is a village with 100 inhabitants, situated 3 miles to the north-east of Mirpur Bathoro, with rice cultivation extending to within 300 yards of its periphery. A minor canal runs close to the village. There are a few borrow-pits at the periphery, in which larvæ of *A. subpictus* were found. Adult specimens of *A. subpictus* and *A. culicifacies* were caught in the village. The splenic index was 38 (26 observations).

GOT SHORA is a village with 50 inhabitants, situated  $4\frac{1}{2}$  miles north-east of Mirpur Bathoro, in the midst of rice cultivation. A minor canal called the Seekha Wah runs about 200 yards of the village. There is no village 'dhand', but there are collections of water draining from the ricefields in excavations near the village. The splenic index was 45 (11 observations only).

JOAKH is a village with a population of 600, situated 7 miles north-east of Mirpur Bathoro in the midst of dry-crop cultivation. To the west of the village is a 'dhand' covering an area of about  $1\frac{1}{2}$  acres, and a minor canal runs within 400 yards of the village. The subsoil water level at the time of the survey was 4 feet. No larvæ were found in the 'dhand', but larvæ of *A. subpictus* were collected from a borrow-pit near it. Adults of *A. subpictus* and *A. culicifacies* in scanty numbers were found in the village. The splenic index was 39 (61 observations), and the parasite index 48 (25 observations).

DARO is a village with 900 inhabitants, situated 5 miles north-west of Mirpur Bathoro. Rice cultivation extends almost to the edge of the village, but owing to the slope of the country most of the water draining from the fields is carried away from it. There were however a few water collections within 400 yards of the village. The Pinyari Canal runs within 100 yards of the village. The subsoil water level was 7 feet at the time of the survey. Larvæ of *A. subpictus* were collected in borrow-pits, and those of *A. pulcherrimus* along the edges of ricefields. Adult specimens of *A. subpictus*, *A. culicifacies* and *A. fuliginosus* were caught in the village. The splenic index was 37 (188 observations) and the parasite index 40 (25 observations).

LAIKPUR is a village with a population of 200, situated in the midst of dry crop cultivation 12 miles north of Mirpur Bathoro. There are two large 'dhands' near the village, and a large borrow-pit close to the Pinyari canal, which runs at a distance of 25 yards from the village. The subsoil water level at the time of the survey was 6 feet. Larvæ of *A. subpictus* were collected



from the borrow-pit, and adults of the same species were caught in the village. The splenic index was 36 (47 observations).

BANO is a village with 460 inhabitants, lying 18 miles north of Mirpur Bathoro, about two miles from a bend of the Indus. The Pinyari Canal takes off from this bend and runs within 300 yards of the village, which lies in the midst of forest land, with scattered patches of dry-crop cultivation. There are a number of excavations and borrow-pits surrounding the village, in which larvæ of *A. subpictus* were found. Larvæ of *A. culicifacies* were found in a small canal distributary. The subsoil water level at the time of the survey was 10 feet. The splenic index was 62 (42 observations) and the parasite index 32 (25 observations).

SABU KHUDAI is a village with 60 inhabitants, situated 3 miles west of Mirpur Bathoro in the midst of rice cultivation. A minor canal runs at a distance of 75 yards from the village. The splenic index was 58 (12 observations only).

SIDIK SHORA is a village with a population of 70, situated 4 miles west of Mirpur Bathoro, in the midst of rice cultivation. There are a number of borrow-pits surrounding the village, and a minor canal runs within 200 yards. Larvæ of *A. culicifacies* and *A. subpictus* were collected from the borrow-pits. The splenic index was 91 (11 observations only).

DARI is a village with 125 inhabitants, situated 5 miles west of Mirpur Bathoro, in the midst of rice cultivation. A minor canal runs within 100 yards of the village. Larvæ of *A. subpictus* were found in two borrow-pits near the village, and those of *A. culicifacies* in the bed of a small canal distributary. The splenic index was 65 (20 observations).

## 2. JATI TALUKA.

This taluka, which has an area of 2,145 square miles, forms the south-eastern portion of Shahbandar Division. It contains 124 villages, with a total population of about 38,000. The portion of the taluka near the coast is a maze of tidal creeks, and further inland there is a saline plain with no cultivation and little vegetation. The country towards the north-east is cultivable, almost the whole of the cultivation being by flow. The principal canals are the Gungro, Saida, Mirza, Sattah and Gungri. The chief crop is rice. The results of spleen examinations made in the taluka are given in Table II.

MUGHULBHIN, the headquarters of the taluka, has a population of 1,750, and is situated on the bank of the Pinyari-Gungro Canal. At the south-western angle of the town a small distributary takes off from the canal and flows eastwards about 40 yards from its periphery. The bank of the canal has been cut in this situation, to allow water to escape into a depression about three-quarters of an acre in area. There are a number of other excavations in the vicinity. There are two tanks covered with rank vegetation, one at the south-eastern

TABLE II.  
Jati Taluka—Results of spleen examinations.

Name of village.	Population.	CHILDREN.				ADULTS.			
		Number examined.	Number with enlarged spleen.	Percentage with enlarged spleen.	A-U measurement of average enlarged spleen (cm.).	Number examined.	Number with enlarged spleen.	Percentage with enlarged spleen.	A-U measurement of average enlarged spleen (cm.).
Mughulbhin .. ..	1,744	122	71	58	77	107	42	39	60
Habib Mullah .. ..	102	20	11	55	88	15	3	20	..
Mullah .. ..	287	32	23	72	91	36	13	36	40
Tar Khawaja .. ..	239	57	6	11	71	23	3	13	..
Sando Bandar .. ..	408	111	53	48	71	33	8	24	70
Tal .. ..	260	69	9	13	83	21	3	14	..
Chubandi .. ..	80	22	1	5	..	17	..	..	..
Memam Sumar .. ..	73	8	2	25	..	4	3	75	..
Tema Mian .. ..	85	23	11	48	86	22	5	23	..
Katiar .. ..	63	21	12	57	78	17	6	35	..
Kado Samejo .. ..	100	20	7	35	56	8	2	25	..
Khairo Jat .. ..	50	12	4	33	..	11	4	36	..
Daho and Geduro .. ..	210	35	16	46	84	21	1	5	..
TOTAL .. ..	3,701	552	226	41	77	335	93	28	55

corner of the town and the other at the north-eastern corner. Beyond the canal in a westerly direction there is rice cultivation, and to the south and east of the town there are a few vegetable gardens, supplied by the distributary alluded to above. There is no cultivation on the northern side of the town. The subsoil water level is about 10 feet.

Larvæ of *A. culicifacies* were collected along the edge of the distributary, in a well close beside this in various borrow-pits, and at the inlet of the depression fed by the distributary. Larvæ of *A. subpictus* were found in borrow-pits

and in the tanks. Adult specimens of *A. subpictus*, *A. culicifacies* and *A. pulcherrimus* were caught in the town. The splenic index was 58 (122 observations) and the parasite rate 52 (25 observations).

HABIB MULLAH is a village with 100 inhabitants, situated in the midst of rice cultivation 6 miles north-east of Mughulbhin. There is a canal distributary flowing within 20 yards of the village, but, owing to the scarcity of water in this, cultivation this year has been limited to the east of the village. Larvæ of *A. culicifacies* were collected from the distributary, and also from a collection of water drained off from a ricefield. Larvæ of *A. subpictus* were found in a borrow-pit. Adult specimens of *A. subpictus*, *A. culicifacies* and *A. pulcherrimus* were caught in the village. The splenic index was 55 (20 observations)

MULLAH is a village with a population of about 300, situated 9 miles north-east of Mughulbhin. The Pinyari Canal flows at a distance 50 yards from its periphery. Between the canal and the village there is an excavation covering about half an acre, which is filled with water from the canal. The subsoil water level is 14 feet. Rice cultivation extends to within 400 yards of the village. Larvæ of *A. culicifacies* were collected at the inlet of the excavation above referred to, and also in two water collections close to the village. Larvæ of *A. subpictus* were found at the edge of the pond, and adults of this species were caught in the village. The splenic index was 72 (32 observations).

TAR KHAWAJA is a village with about 350 inhabitants, situated 7 miles north-east of Mughulbhin in the midst of jungle. There is dry-crop cultivation in the vicinity, and a minor canal runs within 600 yards. No larvæ were found, but adult specimens of *A. subpictus* and *A. pulcherrimus* were caught in the village. The splenic index was 11 (57 observations).

SANDO BANDAR and DUCHO are two villages situated side by side 5 miles south of Mughulbhin, at the junction of Gungro Creek and Sir Creek, with a combined population of 400. It is said that there was an important harbour here before the Sir Creek was silted up. The Gungro Creek receives the tail water of the Pinyari-Gungro Canal, and there is a large swampy area surrounding the villages. Beyond the creek there is rice cultivation. Larvæ of *A. culicifacies* were collected from many places in the swamps and pools, and those of *A. subpictus* were also found. Adult specimens of *A. subpictus*, *A. culicifacies*, *A. pulcherrimus* and *A. fuliginosus* were caught in the villages. The splenic index was 48 (111 observations).

TAL is a village with a population of 260, situated 6 miles south-west of Mughulbhin; rice cultivation extends to within 500 yards of the village. A canal distributary skirts the village, but this is dry usually by the middle of October. Larvæ of *A. culicifacies* were found in the bed of the distributary,

and those of *A. subpictus* in a collection of water drained from the ricefields. The splenic index was 13 (69 observations).

CHUBANDI is a village with 80 inhabitants, situated  $6\frac{1}{2}$  miles south-west of Mughulbhin. There are ricefields at a distance of 600 yards from it, and a small canal distributary runs within 70 yards. No larvæ were found in the vicinity. The splenic index was 5 (22 observations).

MEMAN SUMAR is a village with 75 inhabitants, situated 5 miles south of Mughulbhin. A canal distributary runs within 100 yards of it. No larvæ were found in the vicinity. The splenic index was 25 (8 observations only).

TEMA MIAN is a village of 85 inhabitants, situated half a mile to the south of Mughulbhin, and about 500 yards from the Pinyari-Gungro Canal. A distributary (drying up at the time of the survey) from this runs within 200 yards of the village, and larvæ of *A. culicifacies* were collected in its bed. The splenic index was 48 (23 observations).

KATIAH is a village with 65 inhabitants, situated 2 miles south of Mughulbhin, on the bank of Gungro Creek. Larvæ of *A. culicifacies* were collected in collections of water along the edge of the creek, and those of *A. subpictus* from borrow-pits. The splenic index was 57 (21 observations).

KADO SAMEJO is a village with a population of 100, situated some two furlongs from Mughulbhin. A small canal distributary flows close to the village. The splenic index was 35 (20 observations).

KHAIRO JAT is a hamlet with 50 inhabitants, situated 9 miles west of Mughulbhin. A 'dhand', half an acre in extent, in which larvæ of *A. subpictus* were found, lies within 50 yards of the periphery. There is rice cultivation within 600 yards. The splenic index was 33 (12 observations only).

DAHO and CEDURO are two villages situated close together 10 miles west of Mughulbhin, near a 'dhand' covering about half an acre. There is rice cultivation on the northern aspect, from which water drains into a low-lying area of about half a square mile, in which larvæ of *A. pulcherrimus* were found. Larvæ of *A. culicifacies* were collected from the 'dhand'. The splenic index was 46 (35 observations).

### 3. SHAHBANDAR TALUKA.

This taluka, which has an area of 1,388 square miles is situated at the south-western angle of the division. It contains 104 villages, with a total population of about 40,000. For the most part the soil consists of alluvial loam, with an admixture of sand, but in the extreme south, where the out-flowing water of the Indus meets the in-coming tides of the sea, a deposit of soil takes place which consists of a soft slimy mud, locally named *bhal*, on which rice is grown. The most characteristic feature of the soil generally is that, wherever the silt-laden water of the Indus has ceased to flow over it for a year or two, it turns into 'kalar', and 'kalar' lands again become cultivable when

overflowed for two seasons. The chief canals are the Sattah, Khanto, Ghar and Kodario. The chief crop is rice, and almost all the cultivation is by flow. The results of spleen examinations made in the taluka are given in Table III.

TABLE III.  
Shahbandar Taluka—Results of spleen examinations.

Name of village.		Population.	CHILDREN.				ADULTS.			
			Number examined.	Number with enlarged spleen.	Percentage with enlarged spleen.	A-U measurement of average enlarged spleen (cm.).	Number examined.	Number with enlarged spleen.	Percentage with enlarged spleen.	A-U measurement of average enlarged spleen (cm.).
Chuhar Jamali .. ..	..	927	131	52	40	84	106	37	35	78
Rabdino .. ..	..	70	8	2	25	..	6	..	..	..
Landhi .. ..	..	259	23	4	17	..	22	2	9	..
K. S. Khair Mohamed ..	..	186	13	5	38	..	13	5	38	..
Ladiun .. ..	..	133	37	20	54	77	23	7	30	51
Haider Ali Shah .. ..	..	160	37	20	54	80	22	5	23	82
Pher Kani .. ..	..	125	23	5	21	..	19	5	26	..
Jungo .. ..	..	98	16	7	44	83	21	4	19	..
Shahbandar .. ..	..	457	59	25	42	77	18	5	28	..
Hussain Dal .. ..	..	75	12	4	33	..	12	3	25	..
Pir Karim Shah .. ..	..	80	12	9	75	69	5	3	60	..
TOTAL .. ..	..	2,570	371	153	41	80	267	76	29	70

CHUHAR JAMALI is a village with about 900 inhabitants, situated in the midst of rice cultivation, 5 miles west of Ladiun, the headquarters of the taluka. The Sattah canal runs within 25 yards of the village, and there is a series of borrow-pits along its course. About 100 yards from the south-eastern corner of the village there is an excavation covering an area of quarter of an acre, containing percolation water from the canal. Water draining from the rice-fields collects in depressions to the north and east of the village. The subsoil

water level was 4 feet. Larvæ of *A. subpictus* and *A. pulcherrimus* were found in the various water collections alluded to above, but no larvæ of *A. culicifacies* were found. Adults of all three species were caught in the village. The splenic index was 40 (131 observations) and the parasite index 32 (25 observations).

RADDINO is a village with 70 inhabitants, situated 4 miles west of Ladiun in the midst of rice cultivation. The Sattah Canal runs 50 yards from the village, and a distributary from it within 20 yards. The subsoil water level was 3 feet at the time of the survey. No larvæ were found. The splenic index was 25 (8 observations only).

LANDHI is a village with about 250 inhabitants, situated in the midst of rice cultivation 4 miles west of Ladiun. The Sattah Canal runs within 30 yards of the village. There are a number of borrow-pits in the vicinity, but these were dry at the time of the survey. The splenic index was 17 (23 observations).

KHAN SAHIB KHAIR MOHAMED is a village with about 180 inhabitants, situated one mile west of Ladiun and 300 yards from the Sattah Canal. Rice cultivation extends to within 50 yards of the village. Larvæ of *A. subpictus* were found in some borrow-pits in the vicinity. The splenic index was 38 (13 observations only).

LADIUN, the headquarters of the taluka, is a village with only about 150 inhabitants, on the banks of the Sattah Canal, surrounded by both wet and dry cultivation. There are a number of borrow-pits in the vicinity, in which *A. subpictus* was breeding. Larvæ of *A. culicifacies* were found in a collection of water formed by leakage from a canal distributary. The splenic index was 54 (37 observations).

HAIDER ALI SHAH is a village with 160 inhabitants, situated 6 miles east of Ladiun, 50 yards from the Khanto Canal, in the midst of rice cultivation. Larvæ of *A. culicifacies* were collected from water drained off from the rice-fields, and of *A. subpictus* from borrow-pits. The splenic index was 54 (37 observations).

HUSSAIN DAL is a village with 75 inhabitants, situated on the bank of the Khanto Canal in the midst of rice cultivation. The splenic index was 33 (12 observations only).

PIR KARIM SHAH is a village with 80 inhabitants, situated on the bank of the Khanto Canal in the midst of rice cultivation. The village at the time of the survey was surrounded by collections of water drained off the ricefields, in which larvæ of *A. culicifacies* were found. Larvæ of *A. subpictus* were collected from borrow-pits. Adult specimens of *A. culicifacies*, *A. subpictus* and *A. pulcherrimus* were caught in the village. The splenic index was 75 (12 observations only).

PHER KANI is a village with 125 inhabitants, situated about 120 yards from the Khanto Canal, with rice cultivation extending to within 400 yards of

the periphery. A canal distributary flows within 50 yards of the village. Larvæ of *A. subpictus* were collected from borrow-pits along the course of the distributary. The splenic index was 21 (23 observations).

JUNGO is a village with 100 inhabitants, situated 100 yards from the Khanto Canal. There is rice cultivation within 400 yards of the village. Larvæ of *A. culicifacies* were collected from water draining from the ricefields. The splenic index was 44 (16 observations).

SHAHBANDAR is a village with a population of 450, situated on a slightly elevated site close to the 'Kanhai Dhoru', into which the Khanto and Sattah Canals discharge their tail water. There are swampy areas round the village, and a large pond, in the latter of which larvæ of *A. subpictus* were collected. Adult specimens of *A. subpictus*, *A. pulcherrimus* and *A. culicifacies* were caught in the village. The splenic index was 42 (59 observations). The subsoil water level was 10 feet.

#### 4. SUJAWAL TALUKA.

This taluka, which is situated immediately south-west of Mirpur Bathoro and north-west of Jati talukas, has an area of 269 square miles, and contains 62 villages, with a total population of about 35,000. The most prominent feature of the country is the great extent of the perennial marshes, which fill a chain of depressions running from Wali Shah on the north-west to Sujawal and southwards towards the Gungro Canal, which now conveys the flood water to the sea below Mughulbhin. The soil is the usual alluvial loam of Sind, the deposit of the river Indus. Formerly the taluka was subject to destructive floods from the Indus, but latterly it has been protected by powerful bunds. The chief canals are the Pinyari and Gungro, cultivation being mainly by flow. The principal crop is rice. The results of spleen examinations in the taluka are given in Table IV.

SUJAWAL, the headquarters of the taluka, is a town with a population of about 3,000, situated 4 miles to the east of the Indus. A minor canal branch runs through the centre of the town, but there was only a little water in this at the time of the survey. At a distance of 100 yards from the southern edge of the town there is an irrigation tank covering an area of about one acre, and fed from a canal distributary. There is also a cattle pond close to the northern edge of the town, with rice cultivation near it. Water draining from the ricefields reaches almost to the periphery of the town. The subsoil water level at the time of the survey was 10 feet. Larvæ of *A. culicifacies* were collected from the water collections draining from the ricefields, and from the bed of the canal, and those of *A. pulcherrimus* from the edge of a ricefield. Adult specimens of *A. subpictus*, *A. culicifacies* and *A. pulcherrimus* were caught in the town. The splenic index was 48 (194 observations) and the parasite index 28 (25 observations).

TABLE IV.  
Sujawal Taluka—Results of spleen examinations.

Name of village.	Population.	CHILDREN.				ADULTS.			
		Number examined.	Number with enlarged spleen.	Percentage with enlarged spleen.	A-U measurement of average enlarged spleen (cm.).	Number examined.	Number with enlarged spleen.	Percentage with enlarged spleen.	A-U measurement of average enlarged spleen (cm.).
Mirzo Laghari .. ..	240	42	20	48	8.5	39	13	33	7.3
Bhati .. ..	75	20	14	70	8.8	14	6	43	8.0
Kehran .. ..	125	14	1	7	..	7	1	14	..
Motai Jo Miani .. ..	150	19	2	11	..	16	2	13	..
Behro Sumro .. ..	100	18	5	28	..	12	1	8	..
Kari Mori .. ..	127	33	1	3	..	9	1	11	..
Churetnani .. ..	275	49	29	59	8.0	37	13	35	6.6
Machi .. ..	190	43	13	30	9.3	20	5	25	4.2
Kandri .. ..	150	25	10	40	9.0	20	6	30	7.6
Patori .. ..	90	20	13	65	8.9	9	4	44	..
Belo .. ..	550	128	66	52	8.0	110	41	37	6.4
Soha .. ..	298	35	9	26	8.9	14	2	14	..
Sujawal .. ..	2,071	194	94	48	7.6	93	25	27	7.7
TOTAL ..	5,341	640	277	43	8.1	400	120	20	6.8

MIRZO LAGHARI is a village with 240 inhabitants, situated 7 miles south-east of Sujawal on the bank of a channel called 'Dhoro Nangan', which received surplus irrigation water from the higher areas and discharges it into the Pinyari Canal about a mile from the village. There is rice cultivation within 600 yards of the village, and there are a number of pools and excavations in the immediate vicinity, by the side of the 'dhoro'. The subsoil water level at the time of the survey was 6 feet. Larvæ of *A. culicifacies* were found in a well, and in water collections along the 'dhoro'. In the village adult



specimens of *A. subpictus*, *A. pulcherrimus* and *A. culicifacies* were caught. The splenic index was 48 (42 observations).

BHATI is a village with 75 inhabitants, situated  $2\frac{1}{2}$  miles south of Sujawal, with rice cultivation extending to within 500 yards of it, and a number of borrow-pits in the immediate vicinity. A canal distributary runs within 200 yards of the village. Larvæ of *A. culicifacies* were found in the bed of the distributary, and in collections of water drained off from the ricefields. The splenic index was 70 (20 observations).

KEHRAN is a village with a population of 120, situated in the midst of rice cultivation 3 miles north-east of Sujawal close to the lake known as 'Pinila dhand'. A small canal distributary runs within 50 yards of the village. Larvæ of *A. subpictus* were found in a collection of water draining from the ricefields. The splenic index was 7 (14 observations only).

MOTAI JO MIANI is a village with 150 inhabitants, situated  $3\frac{1}{2}$  miles north-east of Sujawal in the midst of rice cultivation, close to the Pinila dhand. There is a small canal distributary running within 100 yards of the village. No larvæ were found in the vicinity. The splenic index was 11 (19 observations).

BEHRO SUMRO is a village with 100 inhabitants, situated  $3\frac{1}{2}$  miles north-east of Sujawal in the midst of rice cultivation. Larvæ of *A. culicifacies* were found in the bed of a small canal distributary which flows within 39 yards of the village. Adult specimens of *A. subpictus*, *A. pulcherrimus* and *A. culicifacies* were caught in the village. The splenic index was 28 (18 observations).

KARI MORI is a village with a population of about 130, situated  $4\frac{1}{2}$  miles north-east of Sujawal on an embankment thrown across the Pinila dhand. No larvæ were found in the vicinity. The splenic index was 3 (33 observations).

CHURETANI is a village with 275 inhabitants, situated 9 miles north-east of Sujawal in the midst of rice cultivation, within 20 yards of the Pinyari Canal. There are a number of excavations in the vicinity, but most of these were dry at the time of the survey. Larvæ of *A. culicifacies* were found in a borrow-pit near the canal. The splenic index was 59 (49 observations).

MACHI is a village with a population of about 200, situated 8 miles north-east of Sujawal. Rice cultivation extends to within 400 yards of the village. Larvæ of *A. subpictus* were found in borrow-pits in the immediate vicinity. The splenic index was 30 (43 observations).

KANDRI is a village with 150 inhabitants, situated in the midst of rice cultivation 6 miles north of Sujawal. A canal distributary runs within 10 yards of its periphery. Larvæ of *A. culicifacies* were collected from water drained off from the ricefields, and adult specimens of *A. culicifacies*, *A. pulcherrimus* and *A. subpictus* were caught in the village. The splenic index was 40 (25 observations).

PATORI is a village with 90 inhabitants, with rice cultivation extending to within 400 yards of it, and a small canal distributary flowing within 50 yards, in the bed of which larvæ of *A. culicifacies* were found. The splenic index was 65 (20 observations).

BELO is a village with a population of 550, situated 9 miles north of Sujawal, and 2 miles from the Indus, close to a channel called 'Dhoro Nangan'. There are a number of swamps and excavations containing water in the immediate vicinity, and there are ricefields within 600 yards of the village. Larvæ of *A. culicifacies* were found in pools along the 'dhoro', and adult specimens of *A. subpictus*, *A. culicifacies* and *A. pulcherrimus* were caught in the village. The splenic index was 52 (128 observations) and the parasite index 64 (25 observations).

SOHA is a village with a population of about 300, situated 10 miles north of Sujawal. Rice cultivation extends to within 300 yards of the village, and there is a canal distributary running within 400 yards of it. No larvæ were found in the vicinity. The splenic index was 26 (35 observations).

#### BREEDING-PLACES OF ANOPHELINE MOSQUITOES.

Four species of anophelines were encountered in the course of the survey, namely *A. culicifacies*, *A. subpictus*, *A. pulcherrimus* and *A. fuliginosus*, the last named being found in scanty numbers in only a few localities. Of the four species mentioned only *A. culicifacies* is of importance as a malaria carrier, and extensive observations carried out during the last 5 years have proved beyond question that this species is exclusively responsible for the transmission of malaria in Sind. The breeding-places of this species only will therefore be considered here. The chief of these are :—

(a) The beds of canals and of their branches and distributaries, when the water in these is subsiding towards the close of the inundation season.

(b) Water collections caused by leakage from canals and their branches and distributaries. In this connection borrow-pits along their course are of grave importance.

(c) The 'pancho' water which is drained off from the ricefields into any low-lying ground or excavations in the neighbourhood, either forming shallow 'dhands' or filling borrow-pits.

#### RECOMMENDATIONS.

It has been shown that the prevalence of malaria in Shahbandar Division, as in the rest of Sind, is due to the presence and proximity of favourable breeding-places for *A. culicifacies*. The question arises as to whether it is possible to control malaria in the division by attacking these breeding-places. There are 355 villages in the division, and it is obvious that to attempt anti-larval measures in all these villages would be out of the question on economic

grounds. In the case of rural towns with a considerable population, however, the expenditure involved in antilarval measures directed against the principal breeding-places of *A. culicifacies* would be amply repaid by the results obtained. It is suggested that antilarval measures should first be undertaken in the four taluka headquarter towns of the division. Subsequently these measures might be extended to certain other of the larger and more important rural towns and villages.

TABLE V.

*Average monthly attendance at the 4 Taluka Dispensaries of Shahbandar Division per 10,000 population for the years 1928 to 1930.*

Month.	MIRPUR BATHORO.			SHAHBANDAR.			MUGHULBHIN.			SUJAWAL.		
	Attendance per 10,000 of population.			Attendance per 10,000 of population.			Attendance per 10,000 of population.			Attendance per 10,000 of population.		
	1928	1929	1930	1928	1929	1930	1928	1929	1930	1928	1929	1930
January ..	700	633	842	461	308	538	176	171	229	329	306	288
February ..	792	625	942	462	462	615	241	171	376	353	324	318
March ..	992	875	750	461	462	769	135	165	353	335	359	359
April ..	800	592	733	462	538	615	146	159	582	341	382	294
May ..	817	450	667	461	662	461	153	159	541	341	306	280
June ..	683	492	750	385	385	641	141	153	518	353	300	300
July ..	600	567	942	308	308	662	171	171	288	318	276	294
August ..	583	433	975	385	538	846	165	159	242	349	294	347
September	667	483	742	385	846	692	115	141	294	300	359	318
October ..	800	792	958	461	462	308	194	188	229	306	359	353
November	942	1,017	717	661	385	385	177	259	253	282	390	339
December	625	733	817	385	231	385	188	206	200	315	306	194

The measures suggested are as follows :—

(1) Prohibition of rice or other wet cultivation within half a mile of the periphery of the town. *A. culicifacies* does not breed in the ricefields themselves, but there is profuse breeding of this species in the irrigation channels and in the 'pancho' water draining off the fields into any excavations or low-lying ground in the vicinity.

(2) Filling in of borrow-pits and other excavations within the town limits, and within a radius of half a mile from the periphery of the town. Town rubbish may be used for this purpose. Borrow-pits along the course of the canal distributaries are particularly dangerous. Until these have been filled in, water collecting in borrow-pits should be treated with oil or paris green weekly from July 15th to December 15th each year.

(3) The regular weekly treatment of the margins of the canals and their branches and distributaries with paris green within the town limits and from half a mile from the periphery of the town from July 15th to December 15th each year. The beds of canals and irrigation channels from the time when the water begins to subside in them at the close of the inundation season are probably the greatest sources of breeding of *A. culicifacies*, and even if financial considerations should cause operations to be confined to these, the benefit would be very great.

#### *Organization.*

It is impossible to carry out antimalaria measures without intelligent supervision, and it is essential that the person in charge of these should have some previous training. It is suggested that a malaria inspector with 4 coolies should be employed at each taluka headquarters, the work being supervised and checked by the Health Officer of the Division.

Antilarval work with paris green is being carried out during the malaria season each year in the village of Walid, near Larkana, by the Sind Malaria Inquiry, and it is suggested that the malaria inspectors be sent there for about a fortnight to learn the details of the work. This could be arranged with the Officer-in-Charge of the Inquiry. It would be a great advantage if the Health Officer could be sent to attend the 6 weeks annual malaria class held at Karnal, Punjab, by the Malaria Survey of India in March and April. Failing this, arrangements could be made for him to do a short course of training at Walid along with the inspectors. It could probably also be arranged that the Officer-in-Charge, Sind Malaria Inquiry, should visit Shahbandar Division during the malaria season to give advice on antimalaria measures.

#### *Cost of apparatus, paris green, etc.*

It is impossible to give an exact estimate of costs, but the following account of the expenses involved in controlling the breeding of *A. culicifacies* in Walid is given as a guide.

Walid is a village containing about 200 houses, with a population of about 1,000. The chief breeding-places are (1) the bed of the Ghar Canal which runs beside the village, (2) the village 'dhands', and (3) a number of wells.

Antilarval work with paris green in this village as the result of observations on the breeding-places of *A. culicifacies* and the length of the transmission

season is only found necessary for a period of 4 months. The application of these measures has reduced the percentage of adult specimens of *A. culicifacies* collected in the catching stations in the village to 2-5 per cent, as compared with 65-75 per cent in a neighbouring village where there is no control.

The initial expenses were :—

			Rs.	A.	P.
Peerless Dust Gun for paris green	..	..	60	0	0
Haversacks (2)	..	..	5	12	0
Screener for paris green (Butt's)	..	..	5	0	0
Mixer for paris green	..	..	25	0	0
Tin cans (2)	..	..	6	0	0
Tin funnel	..	..	0	12	0
Hand net	..	..	0	14	0
Cups, enamel, and spoons (2 each)	..	..	0	10	0
Well net	..	..	3	0	0
Dishes, photographic (2)	..	..	2	0	0
			109	0	0

The annual recurring expenses are :—

			Rs.	A.	P.
Paris green, 60 lbs.	..	..	47	0	0
Lime, 24 maunds	..	..	21	0	0
			68	0	0

The equipment required for each town or village would be no more than that enumerated in the above list. The annual expense on paris green and lime would of course vary with the extent of the breeding-places in each locality, and also with the length of time during which it is found necessary to adopt control measures. It would probably be in the neighbourhood of about Rs. 200 for the first season, but might be reduced in subsequent years. Standing water in borrow-pits and other excavations may be treated cheaply with waste oil which can usually be obtained from local garages. It is to be noted that the expenditure given above does not include the wages of the staff.

#### *Distribution of quinine.*

It may be asked what can be done to mitigate malaria in the villages in which no antilarval measures are to be attempted. Here the only measure at present available is the provision of increased facilities for the inhabitants to obtain quinine for treatment. In Shahbandar Division, as elsewhere, though quinine is issued for distribution, it is evident that in the majority of cases

it does not reach the people for whom it is intended. In the first place, the amount of quinine received by Medical Officers in charge of dispensaries varies from 4 to 6 lbs. per annum only for each taluka, an insignificant amount for a population of about 35,000. Secondly, although there is a scheme for free distribution of quinine, yet in the localities visited as late as the end of September it was usually found that the school masters and mukhtiarkars had not yet received any quinine for distribution.

It is very necessary that Medical Officers in charge of dispensaries should receive a supply of quinine sufficient for the needs of each taluka, and that quinine should be issued for distribution well before the onset of the annual malaria season. Also that Quinine Registers should be kept by all who receive quinine for distribution, and that such registers be checked by taluka and district officers at frequent intervals.

Thanks are due to the Assistant Director, Public Health, Sind Registration District, Karachi, for placing the services of Mr. Jaithmal, Inspector of Sanitation and Vaccination, Hyderabad, at the disposal of the officer conducting the survey. Also to the Mukhtiarkars of the various talukas, and to Mr. Sugnomal, S.D.O., Karachi Canal District, for rendering all possible help in the course of the survey.

#### SUMMARY.

(1) Shahbandar Division as a whole is highly malarious. The examination of 2,199 children and 1,498 adults in 55 villages showed that 43 per cent of the children and 30 per cent of the adults had enlarged spleens. The Division lies wholly outside the area to be commanded by the Sukkur Barrage Scheme.

(2) Malaria in the division is transmitted by *A. culicifacies*, the chief malaria carrier of Sind. The principal breeding-places of this mosquito are the beds of canals and their distributaries when the water is subsiding in them towards the end of the inundation season, collections of water drained off from ricefields, and water leaking from canals and their distributaries into borrow-pits.

(3) The amount of malaria in any locality is directly proportional to the number and proximity of breeding-places of *A. culicifacies*. Thus, malaria is especially severe in villages situated within 400 yards of canals, creeks and 'dhoros'. Again, villages where water is drained off from ricefields into excavations in the immediate vicinity are highly malarious.

(4) On the other hand the incidence of malaria in villages situated on the banks of large lakes or on embankments built across them is comparatively slight. Thus the hamlets of Kehran, Motai Jo Miani, Behro Sumro and Kari Mori yielded a combined spleen rate of only 10 per cent.

(5) It is recommended that antimalaria measures be undertaken in the four headquarter towns of the division, operations being later extended to other rural towns. The chief measures advocated are the prohibition of wet cultivation in the vicinity of towns, the treatment of the margins of canals and their distributaries with paris green, and the filling in of borrow-pits. It is suggested that the Health Officer of the division shall attend the annual Malaria Course held at Karnal by the Malaria Survey of India, and that Malaria Inspectors should be sent for training to Larkana, where paris green work is being carried out in a village by the personnel of the Sind Malaria Inquiry.

RIVER SAND SILTING AND OTHER ANTIMALARIAL  
MEASURES IN SOUTH SYLHET, ASSAM.

BY

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[September 6, 1931].

FROM the beginning of the British occupation of the Province of Assam, military, agricultural, and industrial operations have been heavily handicapped by the toll that malaria takes. The principal industry, the production of tea, has suffered severely, and the fact that the indigenous population is either sparse, or work-shy, has compelled the importation of practically all labour, thus rendering the disease more exacerbant.

Until very recent years in spite of the ill-health little was done to cope with the problem, and this little almost entirely on the lines of treatment and not prevention: the palliative certainly preventing the depopulation of many estates, but not in the course of years diminishing the endemicity of the disease, nor preventing considerable loss of life. An occasional experiment to try the value of mass-treatment by quinine proved of but slight use. Further, there was no systematic investigation of the epidemiology of the disease, regarding which there prevailed an almost complete ignorance of the local species of *Anopheles*, their 'carrying' capacities, and their habits, an ignorance engendered by the opinion that it was an impossible proposition to mend matters by tackling the insect; and indeed for this supposition some grounds existed, for the climatic conditions and terrain were extremely favourable for insect-life



of all descriptions, and mosquitoes or the breeding-places appropriate to the reputedly dangerous species, were very readily found, apparently everywhere.

Of latter years however the success elsewhere of 'species-sanitation' has become better known, and has encouraged the hope that research would reveal the fact that large areas of the water found on estates might be safely neglected, attention then being concentrated on those parts proved to be dangerous.

Consequently schemes of investigation and control on several groups of estates have been initiated, that under our present consideration being formulated on behalf of Messrs. Duncan Bros. & Co. of Calcutta, who kindly put the estates in their Agency, which lie in two 'Valleys' of South Sylhet, at our disposal for the purpose of seeing what could be done as a practical experiment\*. In the present paper only some estates in one of these valleys, to wit the 'Luskerpore Valley', will be referred to. Subsequent to the inception of the scheme the estates in some other Calcutta Agency Houses joined in, and one of these estates is the subject of part of our report. The executive work has been put in hand slowly, for in view of some costly failures elsewhere in Assam, and some uncertainty as to the capacity of some species as carriers, it appeared desirable to move with caution, and the results at different stages have been carefully checked. The estates moreover being large, and so subdivided into various 'cooly-line' areas and 'out-gardens', it has only been possible to undertake sections at a time.

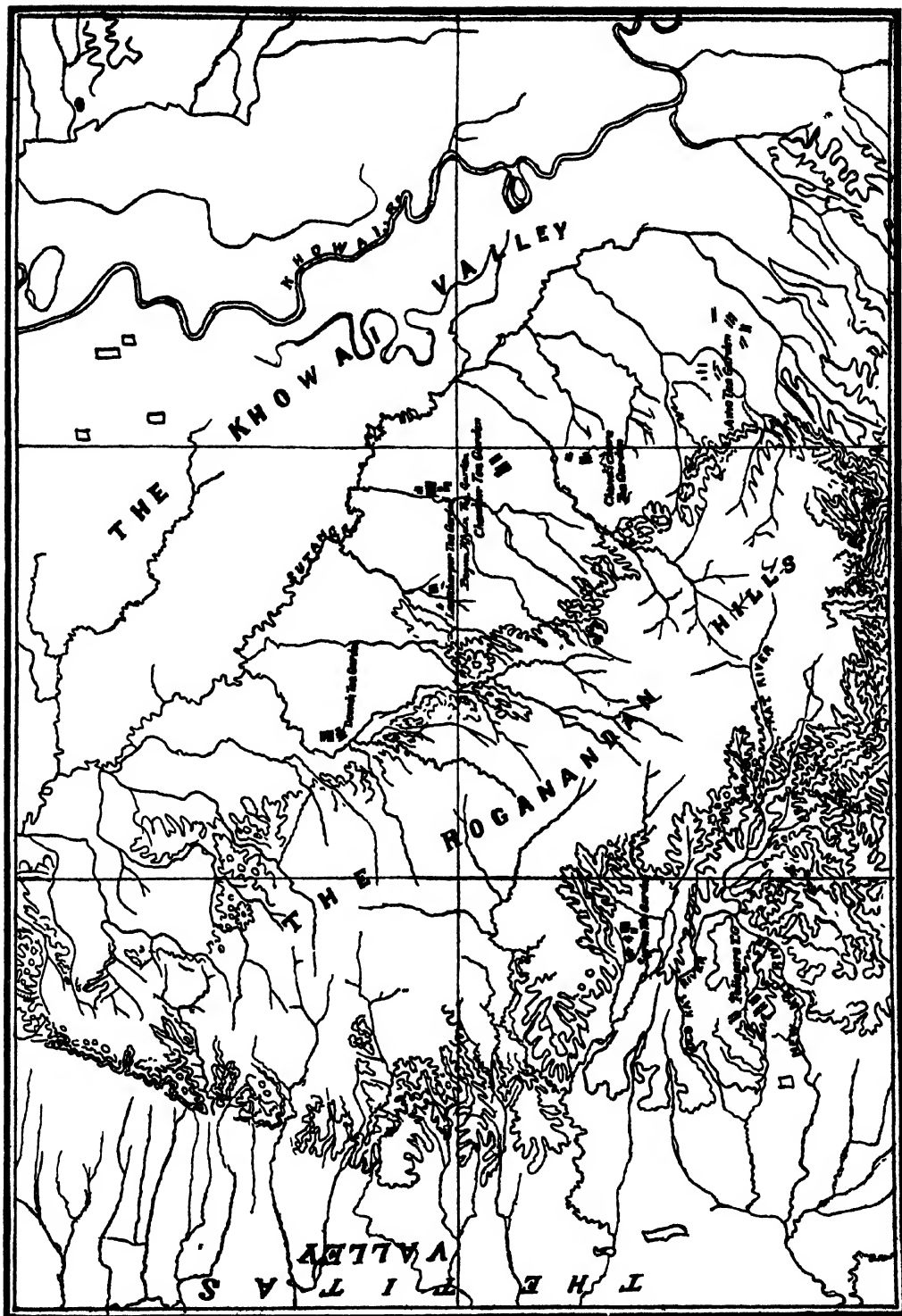
Checks on progress have also been imposed by shortage of the labour that the field-work has called for, and the considerable supervision required, a difficulty now overcome by our having the services of Dr. B. C. Chatterjee as resident malariologist. While it might have been expected that the laboratory work could only be carried out by an assistant with fair capabilities, and after a good deal of tuition, we have also found that spleen-indices have been most untrustworthy unless taken by a trained and proved worker.

### *Physiography.*

The estates dealt with are situated on either side of a very low range of hills where these abut on the recent alluvial flood-plains of the two wide valleys which the range separates; the tea-gardens being mostly planted, and man's habitations built, on the smaller hillocks, the so-called *tilas*. The little valleys between the *tilas* are commonly called *hulas* and these may be at any stage of development or decay, to the extent of the *tilas* being quite surrounded by an alluvial flood-plain and they then appear like islands in a sea. The low-lying land of the flood-plain may be swamped by marsh and mere, (*bhil* land), or, when less water-logged may be cultivated as rice-land, the

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\* Dr. David Gibson was at first associated with us in the work on the estates referred to in this paper, and we are grateful to him for his co-operation.





so-called *pathar* or *dhan-khet* land. The bhils are a prominent feature of this country-side, they are numerous, showing great variations in size and shape, character, and location; exiguous in the foot-hills, expansive on the pathar plain; merely historic sumps where very ancient, but flowing lakes where younger; located upon the banks of tea-land when very old; or upon the pathar, or where the pathar blocks the active hulas, where more recent.

The more recent the bil, the more moving water does it accommodate; the older the bil, the more stagnant and 'tank-like' does it become. Villages on the pathar are often built beside such natural tanks, and of course the rainfall, which is on the average about 100 inches annually, determines the total and seasonal movement of water; in the height of the dry season there is very little moving water, comparatively few streams here being perennial; the hulas are dry, and the bhils stagnant; but during the rains the streams and swamps in every hula flow, and the young bhils fed by them become sheets of slowly-moving water, only the pathar and the old bhils holding up practically stagnant water.

Most of the surface-water in this region is storm-water, but seepage also plays a little part in the supply, and this is best seen in the hulas after the rains.

Long grasses and water-weeds grow profusely everywhere. Only the few active streams are usually scoured clean of vegetation.

#### *Breeding Places.*

It will have been realized then that opportunities for anopheline breeding in this region are excellent, and investigation into the matter confirmed the fact, the following reputed vectors of malaria being prevalent, *funestus*, *aconitus*, *jeyporiensis*, *philippinensis* \* and *culicifacies*.

The bhils were found to be particularly involved, and the problem of how to make them unsuitable for anopheline breeding was no easy one to solve, successful operations as described in other parts of the world affording no help at all in the matter; for we were, it may be said, averse from any measures of a non-permanent nature such as the use of oil or paris-green which had so often been reported successful elsewhere. It was a problem then *sui generis*. In the sequel no one method has been applied to all bhils, several distinct plans to deal with them being evolved after careful study of each particular case. Draining, the most obvious measure, was in many cases not feasible, for lack of a fall, or the fall being too far off to be considered practicable economically, and in those places where it could be used the results had to be very carefully watched, on account of the prevalence of *funestus* and *culicifacies* and allied species which we considered might be correspondingly favoured

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\* This species has not been confirmed as a carrier; in fact in Cachar one of us (C. S.) found no evidence of its reputed pathogenicity.

by this measure. With regard to the latter difficulty however we felt that safety could be insured by growing a riband of jungle over the drains as advised by one of us (C. S.) in 1924 for Assam conditions and this was stipulated for in our recommendations.

In the end the following are the principal methods that we have adopted for dealing with bhils:—

(1) *Silting*.—Where a silt-bearing stream could be diverted through a bhil, the monsoon-floods carried enough silt to convert the marsh into sandy flats, the process a pretty one to observe, and the results ideal.

(2) *Draining*.—An enormous decrease in the breeding areas has been obtained by this measure and that represented a great advantage, but many of the drains, more especially the smaller, have in themselves proved potentially dangerous as we had envisaged, and the overgrowth stipulated for has been for several reasons slow in developing, and consequently it will be some time before the work can be said to be complete. For jungle-cover we have often relied upon the natural growth, but a good deal of planting, chiefly with *Melostoma* spp. has been carried out.

(3) *Bunding*; thus converting marsh into lakes, which it was hoped would be harmless, but unfortunately findings have shown that some small amount of breeding takes place. The prohibition of the use of small-mesh nets is impossible to enforce amongst undisciplined coolies, or the results might have been happier.

(4) *Sumping*; with the intention of converting a large area of shallow water into a limited reservoir. This method has after trial been practically abandoned, the same objection applying as in the preceding method. Both plans however, as a last resort, make some change for the better, in that the 'breeding-edge' is reduced in lineage.

(5) *Training*.—This was of great service in the case of tanks, (and small streams), but in this locality the friable sandy soil, and the absence of stone have been a handicap, and made permanent work difficult. Eventually, where continual attention has been called for, brick-lining for the sides, or a 'pukka' bed will be required.

#### *The terrain of the several gardens.*

Some description in detail now follows of the conditions previously existing on each of three gardens of diverse type, and the measures adopted in them.

AMO ESTATE represented a formidable undertaking, due to the multiplicity and extent of the breeding-grounds of varied nature, such as bhils, hulas, and silt-laden streams. On this estate our findings showed that danger decreased as the hulas approached the open pathar, where malaria was not serious. Owing to the difficult natural conditions on this estate progress in the executive work has been slow, although brilliant, and results not commensurate with the work

carried out. It is only reported here in order to describe the new antimalarial measure utilized in tropical plantations, viz. silting.

The next garden, TELIAPARA, although suffering in the first instance to a much greater degree from malaria than the preceding, shows what can be accomplished under more favourable conditions, the breeding-areas to be dealt with being less extensive.

The third, BEGUM KHAN,\* also demonstrates the value of a preliminary survey as enabling discrimination to be exercised, for findings showed that the extensive water-bearing areas might be neglected, operations being confined to the smaller bhils and the streams, and the first attempt to straighten and clean up the beds of the latter was quickly followed by a decrease in the spleen-indices and an improvement in health.

#### AMO TEA ESTATE.

##### *Physiography in relation to human habitations.*

This estate lies on the abutments of the range on the west of the Khowai Valley, the bed of which is a considerable flood-plain thrown up by the river. The hulas, draining the tilas on which the estate is planted, run down to this flood-plain and are long, narrow and sinuous, drying up early in the hot weather; but during the rains and for a time after they provide the sites of numerous breeding-pools filled with spring-water, which gradually runs away into the fast grassy-edged streams threading their way down the hulas. The tea is mostly grown on the rich high banks of earth, and among the tea here and there are the remnants of old bhils, which are nothing but sumps for rain-water. It is in such a terrain that one cooly-line site has been developed, the New Lines.

As is usual where tributary streams debouch from the hills on to a plain, their regimen has been affected by the mechanical obstruction to the flow of water by the flood-plain of the river that they feed, and 'foot-hill' bhils have been the result. These are expanses of swampy land, from the surface of which is a gentle effluence over the flood-plain: they are maintained as bhils by the scour of heavy rains in the hulas. In the vicinity of this foot-hill bhil-tract two other sets of lines are situated, the Old Lines and the Sonthal Lines.

Another possible factor in the malarial epidemiology of the estate was the presence of some young and active streams traversing it. These streams, coming from forest-denuded land in the hinterland, are heavily silt-laden, and are therefore mostly bare of plants and weeds; and run perennially, although their silted-up beds absorb a great deal of water during the fair-weather flow.

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\* This Estate has been in the sole charge of the junior of us.

*Conclusions regarding the relative danger of different parts of the terrain.*

As the result of our first survey we concluded that the swampy hulas were a great danger owing to the species *culicifacies*, *funestus* and *acomitus*, and that they accounted for the spleen-index of 87 per cent that we obtained in the New Lines. We therefore recommended their contour-draining and that the drains be grown over with jungle.

We also incriminated the foot-hill bhil-tract owing mainly to the presence of the same species, and to deal with this factor Mr. Cruickshank, the manager, undertook to try the measure of silting them up from the rivers, a measure never before, we believe, deliberately tried for antimalarial purposes: the necessary material was at hand in the heavily silt-laden streams spoken of, but it needed Mr. Cruickshank's ingenuity to utilize it as he amazingly did. The plan of the silting-scheme is shown in Map II.

It was a long time afterwards that the silt-bedded active streams were incriminated as malariagenic, and this when *culicifacies* was found breeding in great numbers not only in secluded pools but also over the ripples of alga-matted sand, covered by shallow slow-running water. The mosquito-catching staff having constantly reported them free of breeding-places they had been considered safe, but it is now thought that they may be responsible for some of the malaria at all the sites of habitation on the estate.

We finally investigated the malariagenic possibilities of the flood-plain pathar, that sea of rice-cultivation, and found it to be a comparatively negligible factor, or certainly unnecessary to consider pending the completion of more urgent work.

*The executive operations.*

Mr. Cruickshank has devoted most of his time and resources to the enormous task of the silting-up of the bhils. It has been wonderful to see re-entrants and back-waters silted up, morasses covered as much as six feet deep, silt-bearing channels *en échelon*, others *en échelle*, carrying their sand-burden first in one direction and then in another. But as stated above owing to resources, even of silt-laden water not being limitless, there are still places where the work has not yet been initiated, and other places, some difficult, where it is not yet completed\*. Nevertheless great areas, roughly 50 acres of the original morasses, have been converted into meadows, on which cows may graze with impunity (see Plate V, fig. S).

The consequence as regards the control of mosquito-production of these tracts, where the silting-up has been completed, has been of course the total cessation of breeding, while where the work is incomplete the same breeding as formerly is still going on.

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\* Map II shows the areas not completed by the cold weather, 1929-30.

Some of the bhils have been at too great a distance from the feeder-streams, or it may, for other reasons, have been inconvenient to try to silt them up, and in some of these cases a racquet-shaped drain has been put in; and it remains part of the scheme to cover such drains with jungle. On this estate *Melostoma* spp. (the wild 'rhododendron') have been planted extensively for this purpose. *A. funestus* and *A. culicifacies* have been found breeding in such drains where not covered.

The greater part of the estate resources in time and money having gone, up to date, in the work on the bhils, the draining of the hulas has not yet had the attention designed for them; nevertheless a great deal of work has been done in putting in the contour-drains, and it is jungle-cover for them that has not yet to any appreciable degree been effected, and this is partly due to petty difficulties that will in due course be surmounted.

#### *The results on health.*

These diversified and extensive breeding-areas having called for such considerable operations, progress has of necessity been somewhat slow, and in view of the work not being yet complete, and in fact at the Sonthal Lines site hardly started, one could not have expected any striking improvement in the health. There has however (at the Old Lines site where the greatest effort has been made) been a lowering of the malarial case-rate, the infant death-rate and of the spleen-index.

When one considers what a relatively enormous effort is required to reduce a spleen-index from 100 per cent to 90 per cent as compared with that required for the reduction of one from 10 per cent to nil one may still expect completely successful results at a geometrically increasing rate.

The following data show the main figures involved :—

#### *Approximate population 2,880.*

Name of Lines.	<i>Spleen indices.</i>		
	1925	1930	1932.
Old .. ..	71	51	40
Sonthal .. ..	78	68	70
New .. ..	87	80	66

#### *Number of malarial cases.*

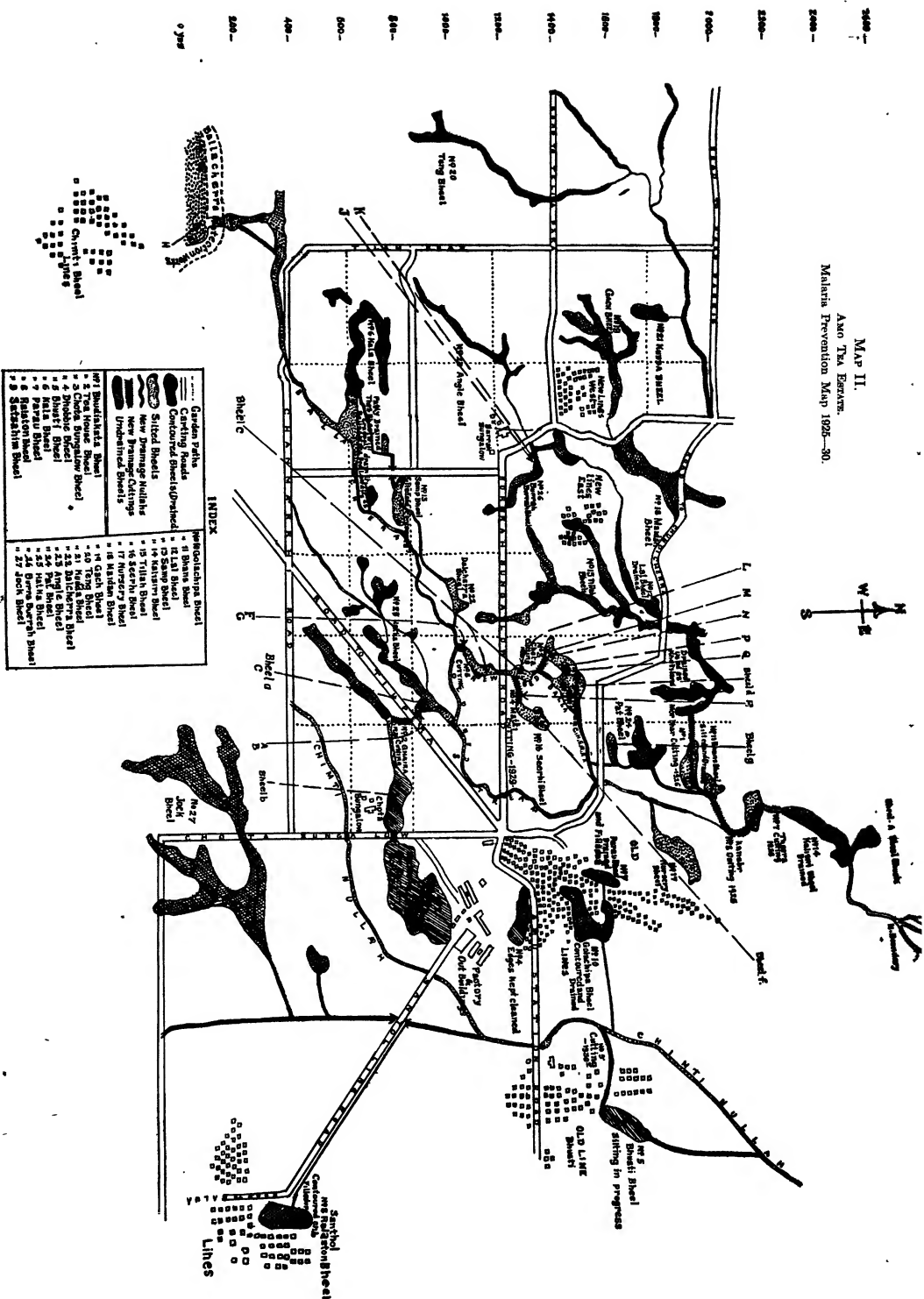
Average for ..	1923-4-5.	1929-30.	1931.
	1,376	881	422

#### *Infant deaths per 100 births*

Average for ..	1924-5-6-7.	1928-29-30
	101	86



MAP II.  
 AMO TKA, FORMER.  
 Malaya Prevention Map 1925-30.



The comparative lack of results at the Sonthal Lines must of course be ascribed to the little work carried out there, as is indicated in the map. We believe that the persistence (1930) of the spleen-index of 40 per cent at the Old Lines may be partly due to the malaria showing an index of 68 per cent at the Sonthal Lines only half-mile away.

*Other results of the operations.*

One good effect of the draining of bhils and hulas has been the complete drying up of the land between the drains, and this has been utilized by the coolies for rice-cultivation or grazing, a new and very valuable asset in all planters' eyes. About 60 acres have been thus reclaimed. The manager Mr. R. Cruickshank to whom we are so heartily indebted for his great interest in the experiment has written to us as follows with regard to the point of view of the estate administration :—

' \* Map II shows the course of the silt-laden stream (the Balucherra) through the garden and the diversions from its course which have been cut to utilize the silt for bhils, which it would have been difficult to drain. We estimate the whole area under treatment to be approximately 75 acres, of which 11 acres have been silted in the course of the Balucherra. Sixty acres have been converted into meadows or plantable land by means of drainage, and siltage carried by garden drains. The area permanently under water, i.e., the Factory and Dhobi bhils, consists roughly of 5 acres which are kept under regular treatment. The general health of the garden has considerably improved, which I attribute to the drainage done in this connection '.

In another letter Mr. Cruickshank has written regarding the benefit accruing from the work at Amo Estate:—' It has generally improved the drainage over an area of at least 150 acres of tea, has made replantable 10 acres which had practically died out as a result of water-logging and made a large number of bhils suitable for the cultivation of padi '.

We have given an account of this estate partly to show the relation of the malaria-incidence to its physiography, but mainly to describe a new and perfect antimalarial method of a permanent nature.

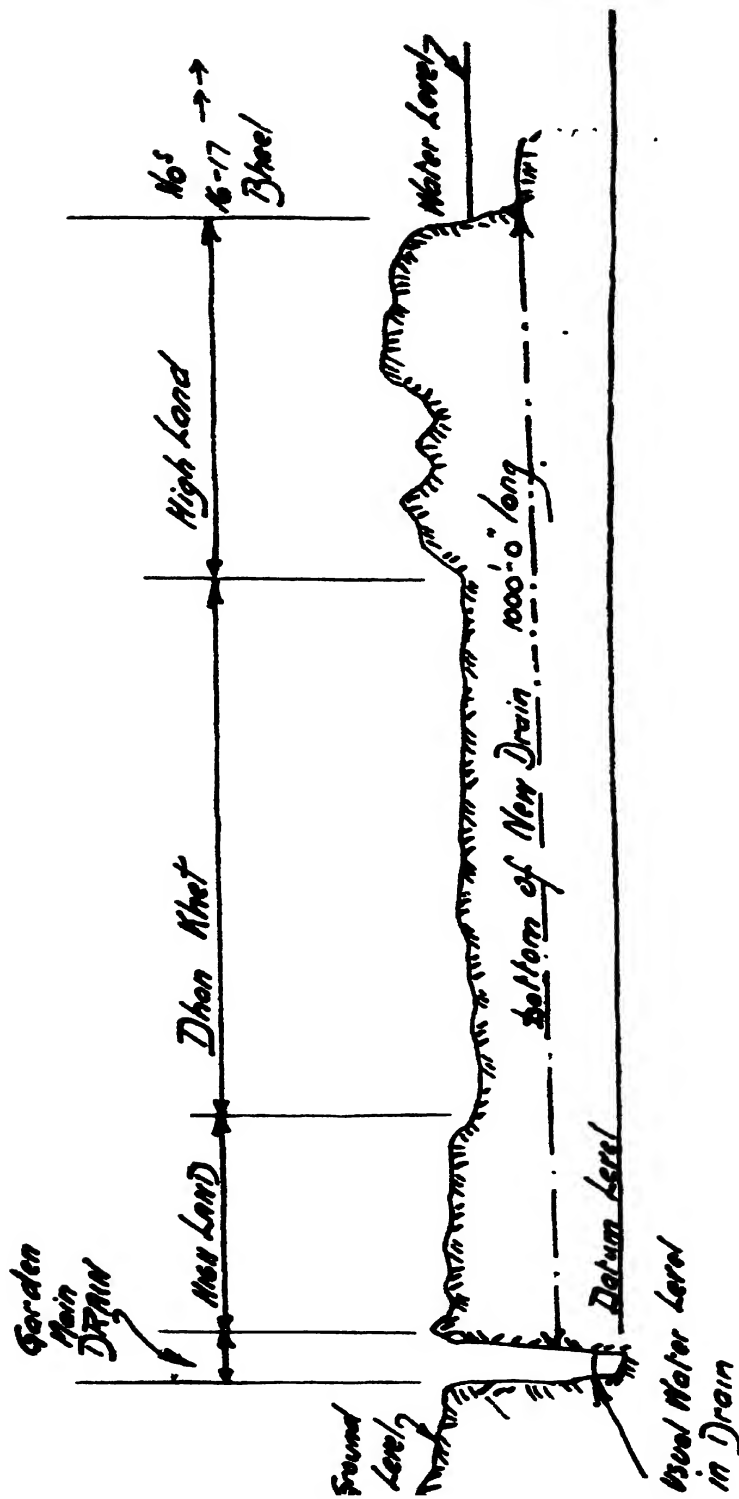
**TELIAPARA TEA ESTATE.**

*Physiography in relation to human habitations.*

Teliapara Estate has the same relation to the Roghanandan range on its west side as Amo Estate has on its east and therefore they are not in same valley as, in common parlance, they are inferred to be. This western side of the hills drains into the Kat River and later into the large river the Titas. Physiographically those estates are very similar with regard to the dissection

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\* For which we are much obliged to Mr. Wilkie.



Sketch 'A'

Showing Profile of Levels between Garden Main Drain and Bhil in Nos. 16 and 17,

Teliapara Tea Estate Antimalaria scheme

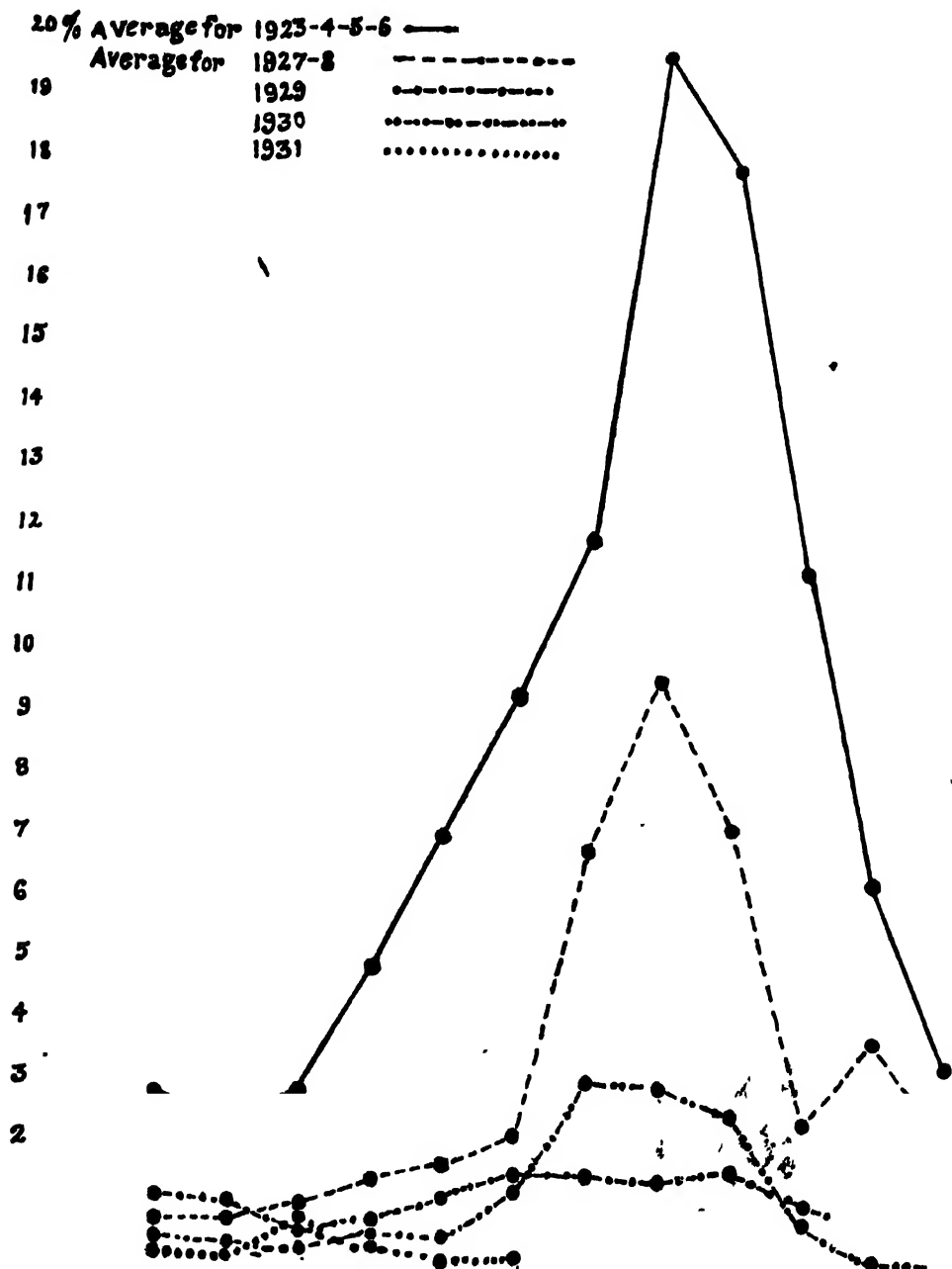
Drawn by G. E. Bates.

*Antimalarial Measures in South Sylhet.*

## TELIAPARA TEA ESTATE

Number of malarial cases monthly, shown in percentage of total population

Jan. Feb. March. Apr. May. June. July. Aug. Sept. Octr. NOVr. Decr.



of the tilas by hula-streams, and the development of bhils at the embouchement of the hulas at the flood-plain of an extensive valley. A point of distinction between them however is that no silt-laden stream traverses Teliapara. The Kat River, a 40-foot stream with a silted bed\*, runs nowhere within the estate except just inside the south-east corner, and it is at the nearest point from human habitations a half-mile away. An old course of this river runs along the northern side of the estate, but is now but little more than a garden drain and is overgrown with jungle.

The lines sites have all been placed near the bhils at the edge of the great valley flood-plain, and none, as are the Amo Estate 'New Lines', in special relation to hulas. The Old Lines particularly are embraced by bhils.

#### *The danger of different parts of the terrain.*

When the malaria survey was first made no breeding was found in the Kat River, and as this was a half-mile away, a distance often stated to be safe, it was thought that it could not be an important factor. It was only subsequently that *culicifacies* was found in the river in large numbers, and it is now considered to be possibly responsible for any malaria there is on the estate.

The extensive bhils were found harbouring many *culicifacies*, and the hulas, which were originally in rather an untidy condition, *funestus* and *aconitus*, so that for executive operations it was decided to attend to the hulas and the bhils.

#### *Executive work.*

Mr. Bates, the manager, for the general purposes of the work, undertook the mapping of the whole estate and its environs, showing all the topographical features and the lay-out of the garden. He also took levels in order to ascertain in what direction to lead the outfall of the garden-drainage.

As a result of his very careful work, he concluded that it was essential here first to lower the outfall at the boundary of the estate, as he could not go outside 'the Grant' to obtain any further fall, a difficulty due to the inadvisability of infringing on other interests; and that has been the key to the work and the results on the estate. The lowering of the outfall was primarily designed to drain out the bhils, for on this estate there was no feasible possibility of bringing in silt-laden water for filling up the bhils as at Amo Estate. Mr. Bates succeeded in lowering the outfall as much as 8 feet, and with this lower level to fall to, he opened the great mass of bhils by cutting through a bank of high land for a distance of 1,000 feet and a depth of 3 to 5 feet.

---

\* On which *culicifacies* breeds prolifically.

Mr. Bates hopes to be able later to obtain a still greater reduction in the level, for even this fall has not been instrumental in draining the bhils dry although the area of water has been enormously reduced.

Where the bed of a bhil has been dried up by drainage or is naturally dry, it is essential to put in shaded hill-foot (contour) drains to carry away seepage from the tilas behind, and such work has been taken in hand. In connection with this however Mr. Bates has written 'in some places contour-drains were put in around the bhils to cut possible seepage but it was found (3 years after) that the subsequent drainage of the bhils left these drains high and dry, due



to the saturation level of the bhil-water being lowered after drainage and consequent sinking of the bhil earth '\*.

*The hulas†.*—For these places jungle-shaded contour-drains were recommended, and this work has proceeded apace.

The greater effort has been expended on getting the bhils dry.

\* Which shows that what had been taken to be possibly seepage water must have been only flood-water for otherwise the drains would not have dried up.

† Mr. Bates describes them as follows:—'Narrow depressions between steep tilas which to some extent always contain water, the water not being stagnant but very nearly so, and the place containing jungle practically throughout'.



This estate up to three years ago was decidedly unhealthy, the general and infantile death-rates being high, and the lengthy sick-lists especially in the leaf-plucking season were of serious concern to the administration.

The figures appended show to some extent the improvement that has been effected, and it is obvious that the estate now enjoys excellent health, even if the vital statistics are judged by European standards; and this is confirmed by the better appearance of the coolies and the few cases of anæmia. It has often been stated that most of the ill-effects of malaria are deep-rooted in the system, but we did not visualize how far-reaching the consequence could be until the amelioration of the condition of the community when saved from the disease was seen in practice.

It is only fair to state that, with the initiation of antimalarial work in 1926-27, a central water supply from an artesian tube-well was installed, so that the whole credit of the improvement in general health cannot be given to the former work, but the reduction in the number of malaria-cases and the spleen-indices show the part that the antimalarial work has played.\*

From a broader point of view the estate is an interesting example of what can be accomplished by a judicious and moderate expenditure on preventive medicine in a sub-tropical climate\*. Where rational antilarval measures are feasible they fully justify themselves, and make a pleasing contrast to the former infructuous distribution of quinine for preventive purposes.

To any one cognizant of the aquatic conditions on the estate during the rainy season, the task of larva reduction might have appeared wellnigh hopeless and it has only been by assiduous study of the material collected in constant survey work that operations could be limited to a practicable extent.

The following figures show the improvement in the health figures of the estate :—

Population 1,340.							
Spleen-indices.					REMARKS.		
			1927.	1930.	1932.		
Old Lines	..	..	74	27	7	The Old Lines come into closer relation with the bhils than the other lines.	
Bich Lines	..	..	87	4	5		
Sonthal Lines	..	..	66	14	8		
Number of cases of malaria (see Chart).							
Average 1923-6	..	..	..	..	..	..	1,284
„ 1927-8	..	..	..	..	..	..	554
„ 1929-30	..	..	..	..	..	..	191
„ 1931	..	..	..	..	..	..	124
Infant deaths per 1,000 births.							
Average 1924-7	..	..	..	..	..	..	148
„ 1928-30	..	..	..	..	..	..	72

\* Assam is not in the Tropics as a writer stated recently.



*Deaths from all causes per 1,000.*

Average 1922-7	..	..	..	..	..	..	29.5
" 1928-30	..	..	..	..	..	..	15.3

*Births per 1,000.*

Average 1922-7	..	..	..	..	..	..	37.0
" 1928-30	..	..	..	..	..	..	44.6

*Average number of daily sick.*

1926	..	..	..	..	..	..	40
1927	..	..	..	..	..	..	43
1928	..	..	..	..	..	..	28
1929	..	..	..	..	..	..	23
1930	.	..	..	..	..	..	19

We now give the point of view of the administration with regard to the work, its difficulties and consequences, and here we would like to congratulate Mr. Bates, the manager, on the execution of the scheme and the fascinating designs of his work.

Mr. Bates, on the above points, has written that there is no doubt but 'that such a well carried-out antimalarial scheme has a very great and beneficial effect on the health of the labour force' insofar as this disease is concerned. At the same time other factors probably have as much as anti-malaria operations to do with maintaining health among coolies, e.g., a good water-supply and good line-sanitation, and these other factors must not be neglected\*. With regard to 'other consequences' from such a drainage scheme as that carried out, there is no doubt, Mr. Bates says, that this may have a bad effect on some *khet* land, leading to dissatisfaction among the holders of the plots, but on the other hand land, which it was formerly impossible to cultivate, is sometimes brought into cultivation. Other administrative difficulties mentioned by Mr. Bates which may crop up are cattle-trespass on the khets, animals breaking down drains, and drains falling in owing to the friability of soil.

The above account we think shows the benefit that can accrue from a scheme indicated by a scientific survey of local malariagenesis and the executive work efficiently carried out by an engineer.

\* In any locality the factors which are important in maintaining the health of the coolies depend of course on what the endemic diseases are. Plague operations are the most important factor in a plague-endemic area and so on. On Teliapara the most important factors in maintaining the health of the labour have been a good water-supply for the endemic bowel diseases and antimalarial work for the hyper-malaria.

Only the antimalarial operations have brought about the great reduction of the spleen-index.

**BEGUM KHAN TEA ESTATE.***Physiography in relation to human habitations.*

The single group of lines on this estate is situated on the slightly undulating land between the Roghanandan hills and the level plain of the valley, the site being bounded on the north and east by the extensive bhils and paddy land of this plain, and on the other two sides by land for the most part planted with tea. Streams course through the garden to the north-east on their way from the hills, while here and there are some small scattered bhils formed by dead or dying water-courses: these streams are an ever-varying quantity, sometimes mere trickles, at other times, after say a fall of eight to ten inches of rain, roaring torrents, over-flowing their banks, and converting the adjoining marshes into lakes.

*The important malariagenic factors on the estate.*

The initial survey of the local malariagenic factors showed that the large bhils and paddy fields to the north-east were, very fortunately, of comparatively negligible importance, otherwise the problem would have assumed much greater dimensions. On the other hand the small bhils and streams in the tea proved to be breeding carrier species freely, the former providing *aconitus*, *funestus*, *philippinensis*, and the latter *aconitus*, *funestus* and *culicifacies*. In comparison with most of the local estates the dangerous area was however fairly small.

*The executive work.*

As for dealing with these places, the streams, running as they do over silty beds and with large seasonable variations in level, are very unmanageable and difficult to make permanently innocuous by inexpensive means, quickly becoming again dangerous with any slackening of attention. Training the banks and keeping them clear of grass, filling in wash-outs, and levelling the beds, have been attended to as far as is possible, but a more permanent solution is much to be desired. A brick or concrete channel would meet the difficulty, as seepage is negligible, but any consideration of this nature must be postponed until the return of more prosperous times.

The small bhils in the tea have been dealt with by draining or filling in.

*Results on health.*

Before the start of operations the garden was decidedly unhealthy, and the high infantile death-rate, low birth-rate and the prevailing fever and anæmia indicated malaria as the cause, this supposition being confirmed by the fairly high spleen-index of 68.

The figures appended show in part the amelioration to the well-being of the coolies that has now been brought about, the infantile death-rate, that sure

barometer of the incidence of malaria, being especially noticeable in the improvement, but they do not give a full representation of the general change for the better that has taken place, nor do they display the increased willingness and capability of the cooly for work as compared with formerly. The coolies themselves largely realize their improved condition, but with Eastern mysticism attribute it to the orientation of the puja house having been altered.

Important, and useful, as the spleen-index is, in malariology, yet to the doctor, and to those to whom he is responsible, the ultimate criterion of success must be the resulting betterment of vital statistics and the sickness rates, and although an index as near zero as possible is the aim of the malariologist, it must not be concluded that the outcome is failure, should the fall be considerably short of what is desired. It is for this reason that this estate has been chosen for this short account, the benefit accruing demonstrating that 'half a loaf is much better than no bread', for here, as has been seen also on other estates, any lowering of the index is accompanied, or more strictly somewhat preceded, by a corresponding improvement in health, and the manner in which the tale of malarial cases moves up and down with even slight alterations in the index is very striking. While still working and hoping for better things it is encouraging to note that the difference between an estate with an index of about 70 and one with from 20 to 30 is from a practical point of view most substantial.

The following data give the most important of the figures involved on this estate :—

<i>Population 940.</i>				
<i>Spleen-indices.</i>				
	1928.		1930.	1932.
	68		31	15
<i>Number of cases of malaria.</i>				
Average 1925-7	..	..	..	663
„ 1928	..	..	..	412
„ 1929-30	..	..	..	223
„ 1931	..	..	..	94
<i>Infant deaths per 1,000 births.</i>				
Average 1924-8	..	..	..	189
„ 1929-30	..	..	..	66
<i>Births per 1,000.</i>				
Average 1922-8	..	..	..	32
„ 1929-30	..	..	..	40
<i>Average number of daily sick.</i>				
1927	..	..	..	22
1928	..	..	..	12
1929	..	..	..	12
1930 ,	..	..	..	14

The manager Mr. Laing has written as follows, showing the point of view of the administration :—

‘ Yearly the incidence of malaria amongst the coolies is becoming smaller. The benefits are many. A healthier and more contented labour force; a higher birth-rate and a lower death-rate. The time when the malarial scourge was at its height, usually coincided with the time when on most gardens every available worker was required.

Now, not only are the workers available, but they are much more able to do their daily task. This can readily be appreciated by anyone who has been the victim of malarial fever, and can recall the before and after-effect of a bout of such.

That these benefits can be proved by actual figures in itself speaks volumes; and when one takes into consideration the fact that this antimalarial work has only been in progress here for three years, it is not too much to expect that within a very few years more, the malarial scourge will be a thing of the past’.

That letter amply acknowledges the fact that on this estate after a sickness-rate at a high steady average for years the antimalarial work was followed in the first year by an enormous change for the better.



PI ATF I



#### EXPLANATION OF PLATE I.

- A. 20th December, 1928, outlet of bhil 'a' (*see* Map II E D C.) which was silted up from the Balucherra before this was diverted at F. The residuum of the silt coming through to bhil 'b' has improved its condition very much.
- B. 24th October, 1927, looking up the cutting into bhil 'a' which was about to be silted up from the Balucherra (its 'old' bed *see* Map).
- C. 12th November, 1927, east side of bhil 'a' looking north at the beginning of operations.
- D. 20th December, 1928, the same operations completed, notice the excellent arable land.
- E. 20th December, 1928, the same bhil but on the west side

#### EXPLANATION OF PLATE II.

- F. Cutting to divert the Balucherra into the system of bails c c c, thus side-tracking the ' Old Balucherra ' (*see* Map).
- G. The mouth of the same cutting seen to the left now scoured out to a lower level than the old course of the river seen up the sloping bank of sand in the centre.
- H. The Balucherra ' protection works ' (*see* Map II to the S. W.). Taken from the outlet side. Behind the bund is the reservoir of water.
- J. 24th October, 1927, the Burra-bungalow bhil looking south at the start of operations. Silting from a small stream.
- K. 12th November, 1927, the Burra-bungalow bhil after silting was completed: bhil now raised well above its former level.



# PLATE II

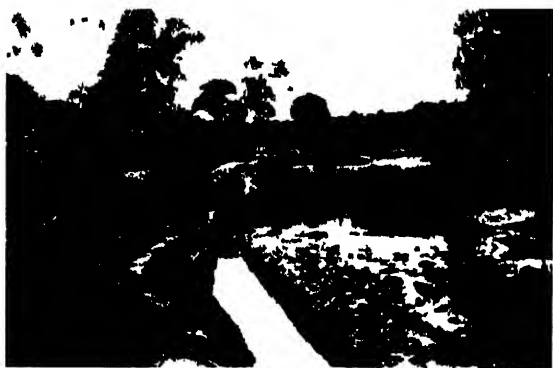


PLATE III



M



N

EXPLANATION OF PLATE III.

- L. 24th October, 1927, bhil 'c' before being silted up by the diversion of the Balucherra at point F (*see* Map).
- M. 1929. Bhil 'd' looking east through the cutting from the Balucherra.
- N. The same.

#### EXPLANATION OF PLATE IV.

- P. 12th November, 1927, bhil 'd' showing silt bank at head of stream (lower down than L).
- Q. 1928, bhil 'd' ('Budhiyahata bhil') before silting up from the Balucherra, now (1931) nearly filled up.
- R. 10th February, 1930, a cutting looking west to silt up a small bhil 'f' to the west of the bhil c c c.

PLATE IV.



Q



PLATE V



EXPLANATION OF PLATE V.

- S. 1930. A former bhil area now a pasture
- T. 1931. A washing-place designed to prevent drains being broken down.





**SOME FINDINGS IN A MALARIA SURVEY CARRIED OUT  
ON A GROUP OF TEA ESTATES IN THE SIBSAGAR  
DISTRICT OF ASSAM FROM AUGUST 1, 1930,  
TO JULY 31, 1931.**

BY

**D. MANSON, M.B., ch.B., L.D.S.,**  
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AND

**G. C. RAMSAY, O.B.E., M.D. (Edin.), D.T.M. & H. (Eng.),**  
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[September 9, 1931]

**INTRODUCTION.**

One of us has recorded the results of his researches in an Anopheline malaria infectivity survey which was carried out on a group of tea estates in the Cachar district of Assam from April 1, 1927, to March 31, 1930, (Ramsay, 1930). The findings in that survey clearly showed that *Anopheles minimus* was practically entirely responsible for the transmission of malaria in the Cachar district of the Surma Valley. An intensive study of the bionomics of *Anopheles minimus* resulted in appropriate methods being devised by which this species is now being efficiently controlled by larvicides, and also, in many instances, eradicated from certain types of breeding areas by biological and other measures. It was essential on this account to have definite and detailed findings regarding the amount of malaria and the bionomics of the species responsible for malaria transmission in the Brahmapootra Valley of the Province, before practical anti-larval measures could be advocated. A survey was therefore carried out, under the auspices of the India Branch of the Ross Institute, on a group of tea estates in the Jorhat Subdivision of the Sibsagar district. These tea estates comprise the Cinnamara Practice and are

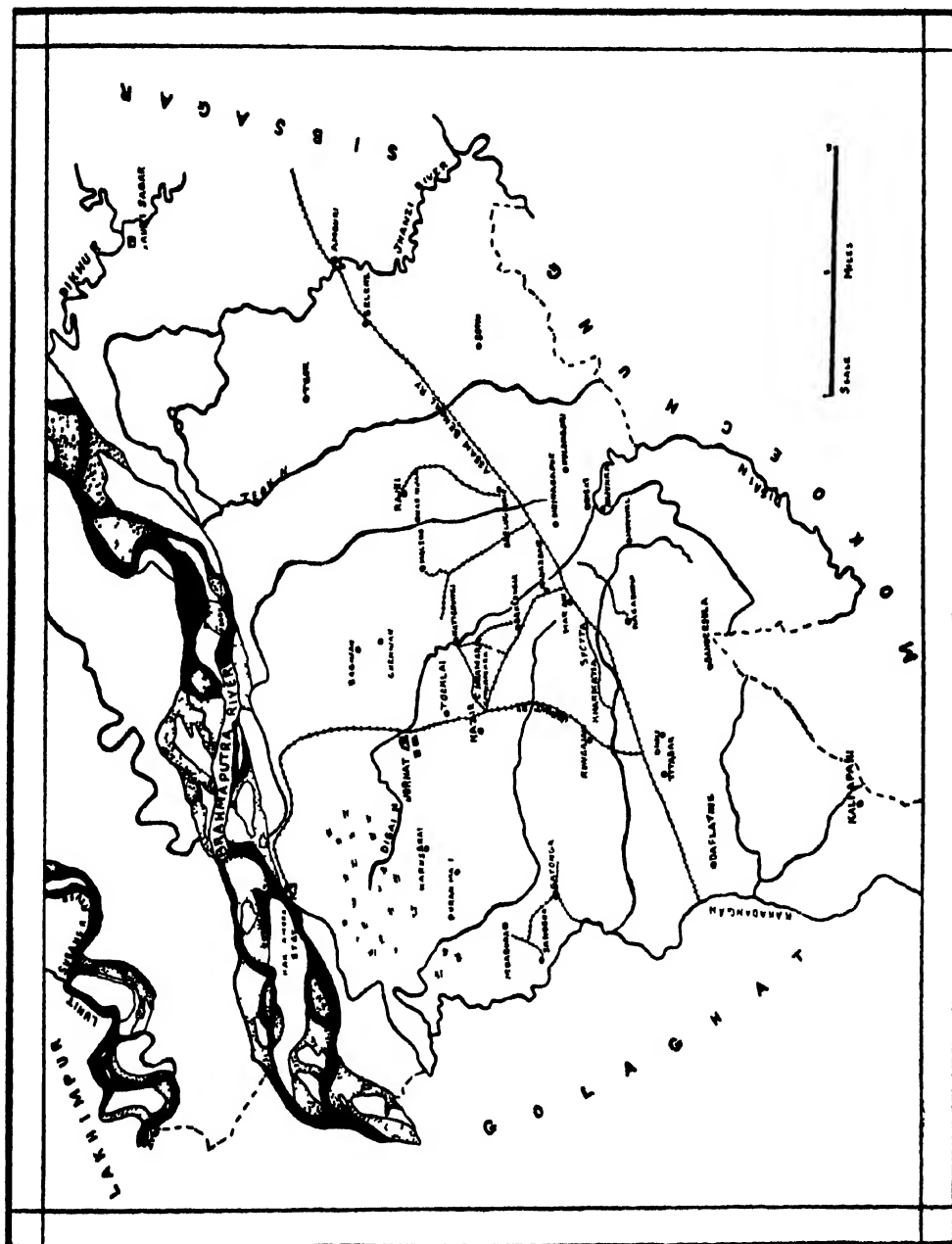
under the medical charge of one of us (D. M.), who has mainly supervised the work carried out in this investigation. This worker has already published some observations relating to the initial stages of this survey (Manson, 1931), also the findings relating to the amount, distribution and effect of malaria on the labour forces in the Cinnamara Medical Practice. The findings and observations which are recorded in this publication are based on a survey extending from August 1, 1930, to July 31, 1931.

#### GEOGRAPHY AND CLIMATOLOGY OF THE DISTRICT.

The Cinnamara Medical Practice is composed of 13 tea estates and is situated in the Jorhat Subdivision of the Sibsagar district of Assam. The Jorhat Subdivision consists of a broad plain rising very gently from the Brahmapootra to the Naga Hills and is about 300 feet above sea level. The plain forms an alluvial tract and is highly fertile, the constituents of the soil being clay, sand and organic matter. The area under survey is mainly devoted to the cultivation of tea and rice. The acreage under tea is approximately 7,000 acres, and the area under rice cultivation is over 2,000 acres. The rest of the land amounting to about 7,000 acres is largely made up of virgin forest and secondary jungle. The district is traversed by several rivers and streams whose general trend is from east to west. Swamps and hullahs or dead rivers are characteristics of the district. The population in the Cinnamara Medical Practice numbers roughly 16,000 souls and, of these, the adult population is engaged in the tea industry.

The scale map shows the Jorhat Subdivision and its boundaries clearly. The actual part of this large area which was surveyed is bounded on the south by the main line of the Assam Bengal Railway between Titabar and Mariani Railway Stations. On the western and eastern sides the boundaries are formed by the two converging branches of the Jorhat Provincial Railway which meet at Cinnamara Railway station and continue as a single line through Jorhat town to the Brahmapootra at Kokilamukh. Part of the area surveyed lies outside this triangle on its eastern side and close to the Dessai River. This outer area comprises three gardens, namely, Cinnamara, Murnuria and Hathichungi, and just above the apex of the triangle, the Tocklai Experimental Station of the Indian Tea Association.

The climate during the year is roughly divided into two seasons—the cold season and the rains; the hot dry season of the rest of India during March, April and May is usually absent due to the fact that there is frequently heavy rainfall in Assam during the north-east monsoon season at a time when precipitation over Upper India is at its minimum. From the beginning of November till the end of February the climate is cool and extremely pleasant, but from May to October it is often very oppressive owing to the high humidity. The mean temperature during the monsoon season averages about



81°F., while during the cold weather it is about 60°F. The average rainfall is about 82 inches, but varies from 66 to 101 inches, the bulk of which falls mainly during the south-west monsoon season.

#### PERSONNEL AND EQUIPMENT.

The personnel in the initial stages of the survey consisted of one Laboratory Assistant and three trained Field-workers, but during the year under review ten additional Field-workers and an additional Laboratory Assistant were trained at the Cinnamara Central Laboratory. Government qualified compounders have been found the most suitable type for training as Field-workers. The average period of training is six months, three of which are spent in the Field and three in the Laboratory. Each tea garden was provided with one Field-worker who was assisted as far as possible by the Hospital Staffs of the various gardens constituting the practice.

Two 'Leitz' and one 'Watson' microscopes, with the usual research equipment, and two 'Leitz' binocular dissecting microscopes were available for research purposes in the Cinnamara Laboratory.

#### FINDINGS AND OBSERVATIONS.

*Larval findings.*—Fourteen species of Anopheline larvæ were collected and identified. The breeding-places of the various species were carefully recorded throughout the year and detailed notes, giving a full description of the physical characters of these breeding-areas, were also made. Scale maps for each tea estate were made showing all the physical features of the land within a radius of half-a-mile from the Coolie Lines.

The following species were collected in their larval form :—

No.	Name of species.
1	<i>A. minimus.</i>
2	<i>A. vagus.</i>
3	<i>A. hyrcanus.</i>
4	<i>A. philippinensis.</i>
5	<i>A. aconitus.</i>
6	<i>A. kochi.</i>
7	<i>A. fuliginosus.</i>
8	<i>A. barbirostris.</i>
9	<i>A. culicifacies.</i>
10	<i>A. maculatus.</i>
11	<i>A. karwari.</i>
12	<i>A. aitkenii.</i>
13	<i>A. tessellatus.</i>
14	<i>A. gigas.</i>

*Adult findings.*—During the year the following species of adult Anophelines were collected, in human habitations and cowsheds, and classified :—

No.	Name of species.
1	<i>A. minimus.</i>
2	<i>A. vagus.</i>
3	<i>A. hyrcanus.</i>
4	<i>A. philippinensis.</i>
5	<i>A. aconitus.</i>
6	<i>A. kochi.</i>
7	<i>A. fuliginosus.</i>
8	<i>A. barbirostris.</i>
9	<i>A. culicifacies.</i>
10	<i>A. maculatus.</i>
11	<i>A. karwari.</i>
12	<i>A. jeyporiensis.</i>
13	<i>A. tessellatus.</i>
14	<i>A. leucosphyrus</i> (only one specimen of this species was collected).

It will be seen from the records that *A. aitkenii* and *A. gigas* were only collected in their larval form while *A. jeyporiensis* and *A. leucosphyrus* were only caught in their adult stage. *A. vagus*, *A. hyrcanus*, *A. philippinensis*, *A. minimus* and *A. kochi* were the most prevalent species both in their larval and adult stages. *A. barbirostris* and *A. fuliginosus* are fairly common in their larval stage throughout the year, but are less prevalent in their adult stage at least in human habitations. *A. aconitus* may be regarded mainly as a cold weather species during which period of the year it is extremely prevalent both in its larval and adult stages, while *A. maculatus* and *A. culicifacies* were chiefly found during March, April and May. Conversely, *Anopheles minimus* was commonly found prevalent in its larval stage throughout the year, but in its adult stage was relatively uncommon during the period—January, February and March. The remaining six species, viz., *A. karwari*, *A. jeyporiensis*, *A. tessellatus*, *A. leucosphyrus*, *A. aitkenii* and *A. gigas* were extremely rare.

The total number of adult Anopheline mosquitoes caught in nature and dissected was 4,296. The mosquitoes were caught practically entirely in collecting tubes from human habitations and cowsheds. Various types of traps were tried, but were not used extensively; the most satisfactory type of the trap which was used was the 'crinoline' trap devised by Richmond and Mendis (1930). The mosquitoes were transferred from collecting tubes to the glass funnels which are used in Dietz lanterns and covered with gauze at the ends. The funnels were then forwarded to the Cinnamara Central Laboratory

and the mosquitoes were kept until they had digested their blood meal. It was found necessary to keep the mosquitoes in a fairly cool room as they rapidly succumbed if kept in a very warm atmosphere. It was also found necessary to keep the collecting jars on a table, the legs of which were immersed in lysol solution to prevent the access of ants which readily prey on adult *Anophelines* in captivity. The number of each species dissected and the malarial findings are recorded in Table I, the results of dissections and the number of the various species dissected during the various months in the year are recorded in Table II to Table XIII.

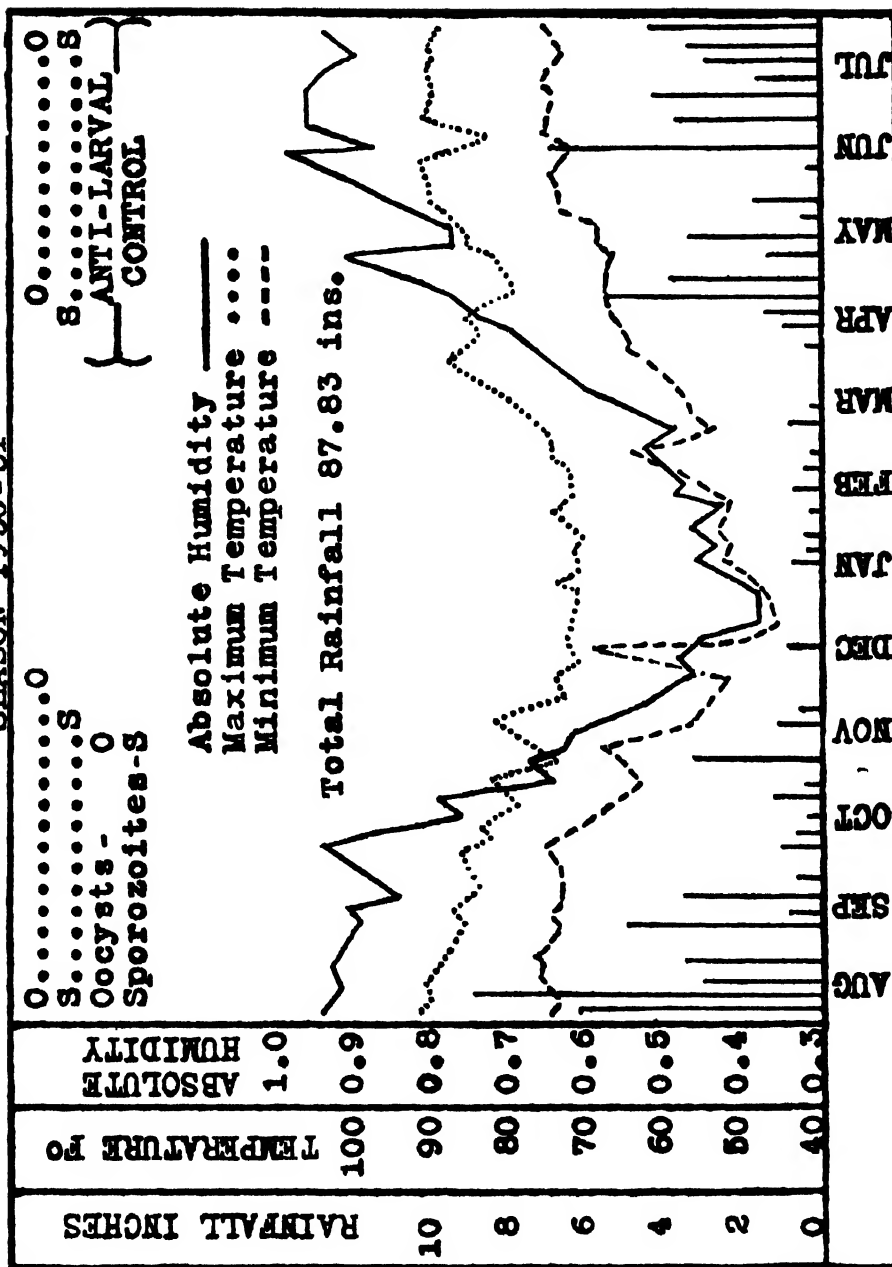
Table XIV shows where the mosquitoes which have been dissected were captured. On Graph 1 is recorded the maximum and minimum temperatures, the vapour pressure or absolute humidity, the rainfall and the period during which mosquitoes were found in nature to be infected with malaria; while Graph 2 shows the relationship of the monthly means of absolute humidity and minimum temperatures to the fever sick rates. From Table I it will be seen that *A. minimus* was the only species found infected in nature. Out of 1,518 dissected, 71 were found infected. This gives a percentage infection of 4.67. The highest rates of infection occurred during October and November, 1930—the percentage infection in October being 7.46, and in November 7.10. During the months of January, February and March, no infections were found. The first infection found in 1931 was on April 17 and the specimen showed sporozoites in the salivary glands. The minimum temperature on April 17 was 66.5°F., the minimum temperatures regularly exceeding 60°F. after March 24. The latest infection was on December 2, 1930. This infection was of the gut only. The minimum temperatures fell below 60°F. on November 22, 1930, and the infection found was most probably a belated survival.

The number of *A. minimus* collected and dissected during April, May, June and July is probably not a true reflex of the numbers which are normally present in this district at that period of the year, as anti-larval measures were directed against *Anopheles minimus* at the beginning of April. Although the staff had been increased to 13 Field-workers, it was found extremely difficult to collect *Anopheles minimus* after these anti-larval measures had been instituted.

From these dissection records it would appear that *A. minimus* is entirely responsible for malaria transmission in the Sibsagar district of Assam. Extended observations, which are now being carried out, will, however, show to what extent other species may be responsible for malaria transmission in this district. As the terrain and climate in the Sibsagar district differ in some respects from that of the Cachar district, we considered that a very thorough study of the bionomics of *A. minimus* in the Sibsagar district was indicated.

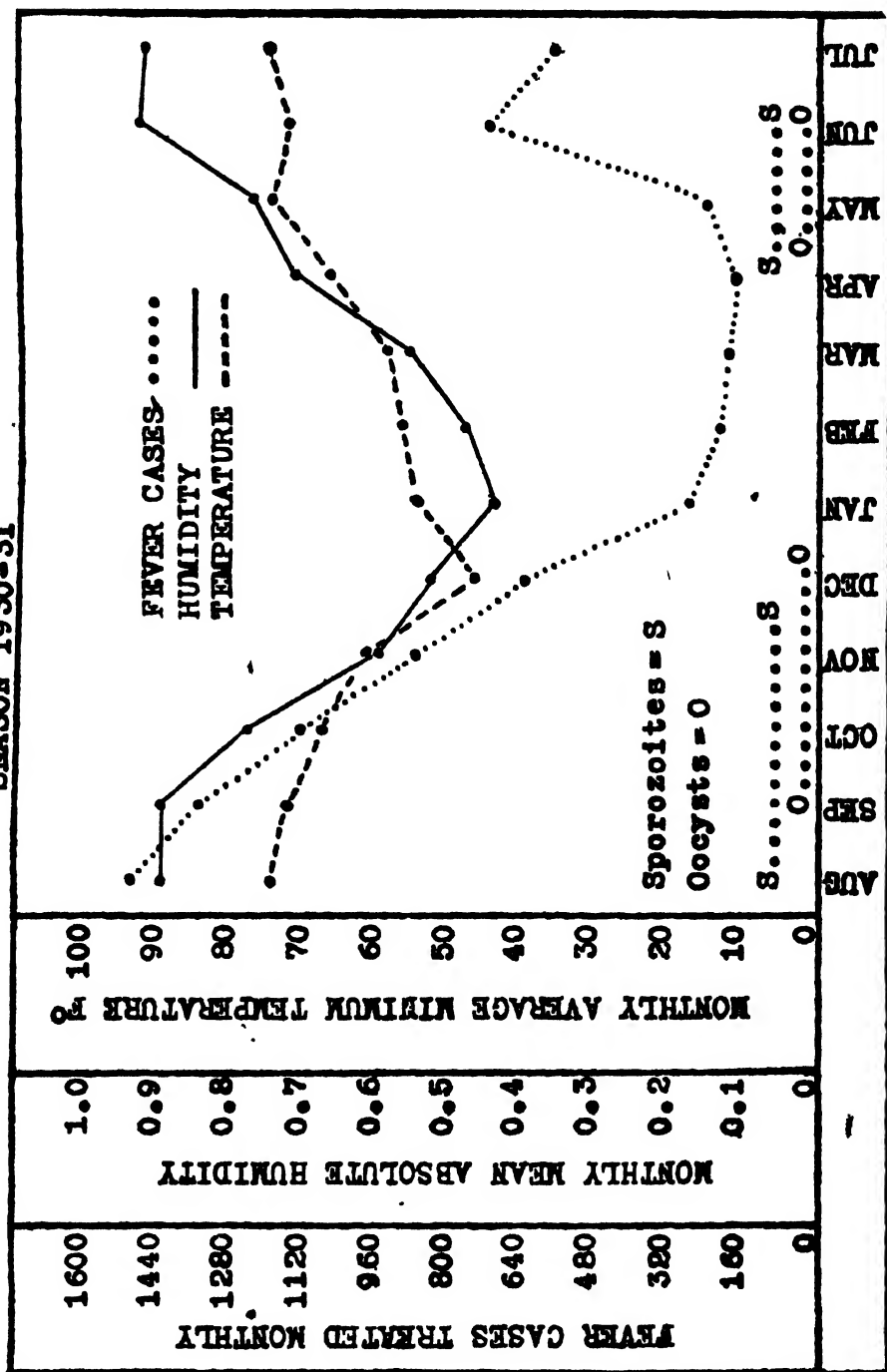
GRAPH 1.

SEASON 1930-31



GRAPH 2.

SEASON 1930-31





BREEDING-PLACES OF *A. minimus*.

*Swamps and seepages.*—*A. minimus* was usually found in these areas. These places usually contained clear running water with a good deal of grass and swamp vegetation and very little shade.

*Hullahs or dead river channels.*—These occur everywhere and are a feature of northern Assam. They usually contain, during the rains, slowly flowing and fairly clear water which is filtered by the vegetation such as "Tarapat" and "Kochu". They contain generally swampy areas and, in places where Tarapat and other shade was deficient, *A. minimus* was generally found.

*Drains.*—Small, narrow, shallow drains (percolators) in tea are generally found free from mosquito larvæ, as these drains rarely retain water sufficiently long enough to allow mosquitoes to complete the aquatic stages of their life cycle. On the other hand, wide, deep, unshaded or partially shaded, clean water drains, in tea sections, generally contains *A. minimus* in association with *A. hyrcanus*, *A. barbirostris*, *A. philippinensis* and *A. vagus* during the monsoon months. During the cold weather months these drains are generally dry.

*Drinking water reservoirs.*—These were found to breed *A. minimus* in association with *A. hyrcanus*, *A. barbirostris*, *A. philippinensis* and *A. aconitus* during the cold weather months and in most cases throughout the year.

*Ricefields.*—Ricefields, which are lying fallow and are close to coolie lines, were found to breed *A. minimus*. When these fields are ploughed up preparatory to the transplantation of the young rice plants, *A. minimus* is no longer found, due, in our opinion, to the larvicidal effect of silt.

*Borrow-pits.*—Borrow-pits when containing silty water are usually free from *A. minimus*, but *A. minimus* has been frequently found in clear water in grassy-edged borrow-pits.

*Rivers.*—Rivers, in conjunction with other perennial waters, were found to be the dry season breeding-places of *A. minimus*. *A. minimus* was found in very large numbers in rivers during the cold weather months and continued to be found in large numbers until these rivers became highly contaminated with silt at the break of the rains. Rivers were also found to contain *A. maculatus*, *A. culicifacies* and *A. aconitus*. There is no doubt that rivers and other perennial streams in this district form an important reservoir for Anophelines. It is also evident that rivers are of great importance in the transmission of malaria during the period between the time when the night temperatures become higher than 60°F. and the time when the rivers become swollen, rapid and silt-laden at the onset of the monsoon. This period varies with the break of the monsoon and particularly with the amount of rain deposited in the Naga Hills as the rainfall in the plains alone does not influence the amount of silt in rivers.

*Bamboo reserves.*—Various bamboo reserves were searched throughout the year to determine the species breeding therein. The only two species commonly

found were *A. hyrcanus* var. *nigerrimus* and *A. barbirostris*, which were found breeding in partially shaded areas. *A. minimus* was never found in these reserves.

*Dense jungle and virgin forest.*—The various densely shaded areas in the district were carefully searched but again *A. hyrcanus* and *A. barbirostris* were the only species found, and these were only found in streams and swamps where there was broken shade admitting a certain degree of sunlight. The search in virgin forest and dense jungle was made particularly carefully for *A. umbrosus* and *A. minimus*, but these species were not found.

In reviewing the characteristics of the breeding-areas in which *A. minimus* was collected, we found that this species selects fairly clear, slowly running or stagnant water, with grassy edges, exposed to sunlight or with very incomplete shade. The importance in certain areas of embankments, narrow culverts, and unsuitable bridges cannot be over-stressed, as these frequently retard the natural flow of water, cause scour-outs to be formed and lead to the loss of natural shade, such as Tarapat.

One of us (D. M.) has found from repeated observations that *A. minimus* is very prevalent in suitable breeding-grounds in close proximity to human habitations, whereas in apparently similar suitable breeding-grounds when remote from human habitations, this species is frequently absent. *A. minimus* was not found in water which contained a high percentage of silt or clay in suspension. It was also absent from water highly contaminated with the products of iron bacteria, and water covered with a thick scum of algal growth. Flushing or high velocity of current in a channel destroys this species as it will all other species of mosquitoes. Finally it was never found in water covered with shade, dense enough to eliminate the sunlight.

We would stress here the importance of silt as an anti-larval factor. Our experience has been that dangerous mosquitoes such as *A. minimus* and *A. culicifacies* will avoid highly silty waters and that, if a high percentage of silt be introduced into the breeding-places of these species, it will destroy their larvæ. By increasing the yield in crops used as food supplies, such as rice, and thereby raising the resistance of the individual, i.e., bonification, silt may be regarded as an indirect anti-malarial factor, but silt, in our opinion, acts practically entirely as a direct anti-malarial measure through its anti-larval action on dangerous species such as *A. minimus* and *A. culicifacies*.

Again the effect of rainfall on the breeding-places of *A. minimus* cannot be over-estimated. In normal years of normal rainfall the perennial streams early in the season become flooded, the velocity of the stream increases and the water is laden with silt. In these years, the perennial streams are found to be free from *A. minimus*, the principal vector of malaria in this district, and they remain free as long as the perennial rivers remain silt-laden. When, however, the rainfall is deficient, the perennial streams are found to contain *A. minimus*

larvæ until much later in the year. As regards the epidemic population of *A. minimus*, in normal years with normal rains the epidemic breeding-grounds are subject to periodic flushes which rid them of Anopheline larvæ. In addition to the factor of flush (velocity of current), many larvæ are drowned during the heavy rainfall which occurs in a normal rainy year. The result is that both from perennial streams where the endemic population reside and from the epidemic breeding-grounds which should normally be flushed, hordes of Anophelines are released in a year of short rainfall or when the rainfall is evenly distributed and there are no heavy downpours.

In conclusion we would point out that on certain types of terrain, mankind, by making drains, borrow-pits, and tanks (drinking water reservoirs), by clearing dense shade from streams and swamps, also in certain areas by making embankments and unsuitable bridges, etc., has unwittingly created ideal breeding-places for *A. minimus*, and is thereby responsible for much needless man-made malaria. It is the duty, therefore, of mankind to rectify, where possible, the errors he has made.

#### ADULT HABITS.

In its adult stage *A. minimus* in nature has a decided predilection for human blood. Table XIV shows that of 1,518 *A. minimus* caught in nature 1,493 or 98.3 per cent were caught in human habitations, while only 25 or 1.7 per cent were caught in cowsheds. The fact that a mosquito is caught in a human habitation, of course, does not mean that it has fed there and *vice versa* as far as cowsheds are concerned, but in the absence of tests to determine the source of the blood meal (precipitin tests) the only criterion which was available to us of species habit was to compare the actual numbers caught in human habitations and cowsheds. Table XIV would indicate that in the Sibsagar district *A. vagus* was more prone to visit human habitations than cowsheds. This finding is evidently variable as in Cachar over 83 per cent of the *A. vagus* collected were caught in cowsheds.

The difficulty in finding *A. minimus* in its adult stage during the cold weather months was, in our opinion, mainly due to the effect of temperature. When the night temperatures fall below 60°F., it is extremely easy to collect *A. minimus* in its larval stage in its winter breeding resorts, but in its adult form it is very scarce. Temperature directly affects the feeding stimulus of adult *A. minimus* as during the cold weather months *A. minimus*, which had fed, were found chiefly in bungalows and hospitals which were heated with fires. It was also found gorged with blood during the warm day-time in dark coolie huts. Unfed specimens were usually collected from unheated dwellings. Graph 2 clearly shows the relationship between fever sick rates and minimum

temperatures. The diminution of malarial sickness during the cold weather is due, in our opinion, to several factors:—

- (1) To the greatly decreased numbers of *A. minimus* which frequent human habitations.
- (2) To their greatly diminished activity in feeding due to low night temperatures.
- (3) To the increased resistance of the individual, especially those partially immune.

The dissection records clearly show in Sibsagar, as they did in Cachar, that October and November are the months when malaria transmission has reached its peak point. It is our opinion that much of the malaria contracted during that period of the year lies latent, especially in partially immunes, until the arrival of the hotter months when there are greater demands on the physical reserves of the individual.

#### SUMMARY.

(1) *A. minimus* was the only Anopheline mosquito found infected in the Sibsagar district during the survey which was carried out from August 1, 1930, to July 31, 1931, but further investigations are being carried out to determine the degree of possible infectivity of other local Anopheline species.

(2) An intensive study of the bionomics of *Anopheles minimus* shows that this species breeds in fairly clear water, slowly running or stagnant, with grassy edges exposed to sunlight or under partial shade only, and that it does not breed in water highly contaminated with silt, clay in suspension, iron oxide bacteria, or water thickly covered with a scum of surface algæ; also that it does not breed in water covered with dense shade or in streams or channels where there is a high velocity of current, except in bays and pockets in these channels.

(3) That perennial rivers and streams are endemic breeding-grounds of *Anopheles minimus* during the cold weather months and until the onset of the monsoon when these water courses become highly silt-laden.

(4) That ricefields, which are lying fallow and in close proximity to human habitations, are potential breeding-places of *A. minimus* until the land is cultivated.

(5) That tanks (drinking water reservoirs) on terrain where the water remains clear during the malaria transmission season are highly malariogenic.

(6) That drains on tea estates which carry clear water are ideal breeding-places of *Anopheles minimus*.

(7) That silt as an anti-malarial factor acts practically entirely through its anti-larval action on *A. minimus*, and not indirectly by increasing the yield in crops (bonification).

(8) That some bridges and culverts which dam the natural flow of water frequently create ideal conditions for the breeding of *Anopheles minimus*.

(9) That *Anopheles minimus* has a decided predilection for human blood and that zoo-prophylaxis plays a considerable part in regulating the feeding habits of most other Anopheline species.

(10) That, in the Sibsagar district, temperature by inhibiting the feeding stimulus of *Anopheles minimus* is a limiting factor in the transmission of malaria.

(11) That the highest infection rate of *A. minimus* was found during October and November and that although much malarial transmission takes place during this period of the year, the infection, at any rate, in partially immune human beings, lies latent during the cold weather months and becomes manifest when the physical resistance is lowered by climatic conditions during the hot weather months.

(12) That mankind on certain types of terrain, where the water remains clear during the malaria transmission season, by making drains, borrow-pits, and tanks, by constructing narrow bridges and culverts which interfere with the natural drainage of water and by clearing dense shade from streams and swamps has unwittingly created ideal breeding-places for *Anopheles minimus*, and is thereby responsible for much needless man-made malaria. It is the duty, therefore, of mankind to rectify, where possible, the errors which he has unfortunately made.

#### ACKNOWLEDGMENTS.

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We have also to thank the Scientific Staff of the Tocklai Experimental Station, Jorhat, for much help and advice during our researches.

We also wish to record here our appreciation of the help and co-operation we have received from Mr. A. Locket, Supervisor of the Jorehaut Tea Company, and the Managers of the various tea estates in the Cinnamara Medical Practice.

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## NOTE.

Since this paper was sent for publication we have succeeded in finding *A. umbrosus*.

This species was found breeding outside the controlled areas in stagnant peaty water, under fairly good shade but with intermittent patches of sunlight. The conditions were inimical to *A. minimus* but suitable for *A. aithenii*, *A. leucosphyrus*, *A. barbirostris*, and *A. hyrcanus*.

An intensive study of the bionomics of this species is now being carried out.

TABLE I.

*Anopheline mosquitoes dissected from August 1, 1930, to July 31, 1931.*

Number.	Name of species.	Number dissected.	RESULTS.	
			Glands infected.	Gut infected.
1	<i>A. minimus</i> .. ..	1,518	40	31
2	<i>A. vagus</i> .. ..	795	..	..
3	<i>A. hyrcanus</i> .. ..	774	..	..
4	<i>A. philippinensis</i> .. ..	462	..	..
5	<i>A. aconitus</i> .. ..	391	..	..
6	<i>A. kochi</i> .. ..	142	..	..
7	<i>A. fuliginosus</i> .. ..	109	..	..
8	<i>A. barbirostris</i> .. ..	87	..	..
9	<i>A. culicifacies</i> .. ..	6	..	..
10	<i>A. maculatus</i> .. ..	5	..	..
11	<i>A. karwari</i> .. ..	3	..	..
12	<i>A. jeyporiensis</i> .. ..	2	..	..
13	<i>A. tessellatus</i> .. ..	2	..	..
TOTAL ..		4,296	40	31

TABLE II.

*Anopheline mosquitoes dissected during the month of August, 1930.*

Number.	Name of species.	Number dissected.	RESULTS.	
			Glands infected.	Gut infected.
1	<i>A. minimus</i> .. ..	39	1	..
2	<i>A. hyrcanus</i> .. ..	47	..	..
3	<i>A. vagus</i> .. ..	33	..	..
4	<i>A. philippinensis</i> .. ..	32	..	..
5	<i>A. kochi</i> .. ..	6	..	..
6	<i>A. fuliginosus</i> .. ..	5	..	..
7	<i>A. barbirostris</i> .. ..	4	..	..
8	<i>A. karwari</i> .. ..	1	..	..
TOTAL ..		167	1	..

TABLE III.

*Anopheline mosquitoes dissected during the month of September, 1930.*

Number.	Name of species.	Number dissected.	RESULTS.	
			Glands infected.	Gut infected.
1	<i>A. minimus</i> .. ..	349	1	6
2	<i>A. vagus</i> .. ..	99	..	..
3	<i>A. philippinensis</i> .. ..	38	..	..
4	<i>A. hyrcanus</i> .. ..	37	..	..
5	<i>A. fuliginosus</i> .. ..	3	..	..
6	<i>A. jeyporiensis</i> .. ..	2	..	..
7	<i>A. kochi</i> .. ..	2	..	..
8	<i>A. karwari</i> .. ..	1	..	..
9	<i>A. barbirostris</i> .. ..	1	..	..
	TOTAL ..	532	1	6

TABLE IV.

*Anopheline mosquitoes dissected during the month of October, 1930.*

Number.	Name of species.	Number dissected.	RESULTS.	
			Glands infected.	Gut infected.
1	<i>A. minimus</i> .. ..	362	18	9
2	<i>A. vagus</i> .. ..	65	..	..
3	<i>A. philippinensis</i> .. ..	42	..	..
4	<i>A. hyrcanus</i> .. ..	29	..	..
5	<i>A. kochi</i> .. ..	5	..	..
6	<i>A. barbirostris</i> .. ..	2	..	..
7	<i>A. fuliginosus</i> .. ..	1	..	..
8	<i>A. aconitus</i> .. ..	1	..	..
	TOTAL ..	507	18	9

TABLE V.

*Anopheline mosquitoes dissected during the month of November, 1930.*

Number.	Name of species.	Number dissected.	RESULTS.	
			Glands infected.	Gut infected.
1	<i>A. minimus</i> .. ..	394	18	10
2	<i>A. aconitus</i> .. ..	41	..	..
3	<i>A. hyrcanus</i> .. ..	40	..	..
4	<i>A. vagus</i> .. ..	35	..	..
5	<i>A. philippinensis</i> .. ..	33	..	..
6	<i>A. kochi</i> .. ..	9	..	..
7	<i>A. barbirostris</i> .. ..	1	..	..
	TOTAL ..	553	18	10

**TABLE VI.**  
*Anopheline mosquitoes dissected during the month of December, 1930.*

Number.	Name of species.	Number dissected.	RESULTS.	
			Glands infected.	Gut infected.
1	<i>A. minimus</i> .. ..	77	..	1
2	<i>A. aconitus</i> .. ..	133	..	..
3	<i>A. philippinensis</i> .. ..	83	..	..
4	<i>A. hyrcanus</i> .. ..	53	..	..
5	<i>A. vagus</i> .. ..	17	..	..
6	<i>A. kochi</i> .. ..	11	..	..
7	<i>A. fuliginosus</i> .. ..	3	..	..
8	<i>A. barbirostris</i> .. ..	3	..	..
9	<i>A. barwari</i> .. ..	1	..	..
	TOTAL ..	381	..	1

**TABLE VII.**  
*Anopheline mosquitoes dissected during the month of January, 1931.*

Number.	Name of species.	Number dissected.	RESULTS.	
			Glands infected.	Gut infected.
1	<i>A. minimus</i> .. ..	15	..	..
2	<i>A. aconitus</i> .. ..	123	..	..
3	<i>A. hyrcanus</i> .. ..	66	..	..
4	<i>A. philippinensis</i> .. ..	36	..	..
5	<i>A. kochi</i> .. ..	8	..	..
6	<i>A. vagus</i> .. ..	4	..	..
7	<i>A. barbirostris</i> .. ..	3	..	..
8	<i>A. fuliginosus</i> .. ..	2	..	..
	TOTAL ..	257	..	..

**TABLE VIII.**  
*Anopheline mosquitoes dissected during the month of February, 1931.*

Number.	Name of species.	Number dissected.	RESULTS.	
			Glands infected.	Gut infected.
1	<i>A. minimus</i> .. ..	2	..	..
2	<i>A. hyrcanus</i> .. ..	61	..	..
3	<i>A. philippinensis</i> .. ..	20	..	..
4	<i>A. aconitus</i> .. ..	18	..	..
5	<i>A. barbirostris</i> .. ..	5	..	..
6	<i>A. vagus</i> .. ..	2	..	..
7	<i>A. fuliginosus</i> .. ..	1	..	..
	TOTAL ..	109	..	..



TABLE IX.  
Anopheline mosquitoes dissected during the month of March, 1931.

Number.	Name of species.	Number dissected.	RESULTS.	
			Glands infected.	Gut infected.
1	<i>A. minimus</i> .. ..	14	..	..
2	<i>A. hyrcanus</i> .. ..	43	..	..
3	<i>A. philippinensis</i> .. ..	31	..	..
4	<i>A. aconitus</i> .. ..	19	..	..
5	<i>A. barbirostris</i> .. ..	3	..	..
6	<i>A. kochi</i> .. ..	2	..	..
7	<i>A. vagus</i> .. ..	1	..	..
TOTAL ..		113	..	..

TABLE X.  
Anopheline mosquitoes dissected during the month of April, 1931.

Number.	Name of species	Number dissected.	RESULTS.	
			Glands infected.	Gut infected.
1	<i>A. minimus</i> .. ..	111	1	2
2	<i>A. hyrcanus</i> .. ..	113	..	..
3	<i>A. vagus</i> .. ..	63	..	..
4	<i>A. aconitus</i> .. ..	45	..	..
5	<i>A. philippinensis</i> .. ..	43	..	..
6	<i>A. kochi</i> .. ..	19	..	..
7	<i>A. fuliginosus</i> .. ..	19	..	..
8	<i>A. barbirostris</i> .. ..	14	..	..
9	<i>A. culicifacies</i> .. ..	4	..	..
10	<i>A. maculatus</i> .. ..	2	..	..
TOTAL ..		433	1	2

TABLE XI.  
Anopheline mosquitoes dissected during the month of May, 1931.

Number.	Name of species.	Number dissected.	RESULTS.	
			Glands infected.	Gut infected.
1	<i>A. minimus</i> .. ..	101	..	2
2	<i>A. vagus</i> .. ..	155	..	..
3	<i>A. hyrcanus</i> .. ..	133	..	..
4	<i>A. kochi</i> .. ..	42	..	..
5	<i>A. fuliginosus</i> .. ..	38	..	..
6	<i>A. barbirostris</i> .. ..	31	..	..
7	<i>A. philippinensis</i> .. ..	17	..	..
8	<i>A. maculatus</i> .. ..	3	..	..
9	<i>A. culicifacies</i> .. ..	2	..	..
10	<i>A. tessellatus</i> .. ..	1	..	..
11	<i>A. aconitus</i> .. ..	1	..	..
TOTAL ..		524	..	2

TABLE XII.

*Anopheline mosquitoes dissected during the month of June, 1931.*

Number.	Name of species.	Number dissected.	RESULTS.	
			Glands infected.	Gut infected.
1	<i>A. minimus</i> .. ..	41	1	1
2	<i>A. vagus</i> .. ..	113	..	..
3	<i>A. hyrcanus</i> .. ..	93	..	..
4	<i>A. philippinensis</i> .. ..	44	..	..
5	<i>A. barbirostris</i> .. ..	19	..	..
6	<i>A. fuliginosus</i> .. ..	16	..	..
7	<i>A. kochi</i> .. ..	13	..	..
8	<i>A. aconitatus</i> .. ..	7	..	..
	TOTAL ..	346	1	1

TABLE XIII.

*Anopheline mosquitoes dissected during the month of July, 1931.*

Number.	Name of species.	Number dissected.	RESULTS.	
			Glands infected.	Gut infected.
1	<i>A. minimus</i> .. ..	13	..	..
2	<i>A. vagus</i> .. ..	208	..	..
3	<i>A. hyrcanus</i> .. ..	59	..	..
4	<i>A. philippinensis</i> .. ..	43	..	..
5	<i>A. kochi</i> .. ..	25	..	..
6	<i>A. fuliginosus</i> .. ..	21	..	..
7	<i>A. aconitatus</i> .. ..	3	..	..
8	<i>A. barbirostris</i> .. ..	1	..	..
9	<i>A. tessellatus</i> .. ..	1	..	..
	TOTAL ..	374	..	..

TABLE XIV.

*This table shows where the mosquitoes which have been dissected were captured during the year under review.*

Number.	Name of species.	Caught from human habitations.	Caught from cowsheds.	TOTAL.
1	<i>A. minimus</i> .. ..	1,493	25	1,518
2	<i>A. vagus</i> .. ..	537	258	795
3	<i>A. hyrcanus</i> .. ..	272	502	774
4	<i>A. philippinensis</i> .. ..	215	247	462
5	<i>A. aconitatus</i> .. ..	80	311	391
6	<i>A. kochi</i> .. ..	58	84	142
7	<i>A. fuliginosus</i> .. ..	47	62	109
8	<i>A. barbirostris</i> .. ..	15	72	87
9	<i>A. culicifacies</i> .. ..	4	2	6
10	<i>A. maculatus</i> .. ..	2	3	5
11	<i>A. karwari</i> .. ..	2	1	3
12	<i>A. jeyporiensis</i> .. ..	2	..	2
13	<i>A. tessellatus</i> .. ..	1	1	2

## CONFIRMATION OF *A. PHILIPPINENSIS* AS A MALARIA CARRIER IN BENGAL.

BY

K. BOSE,

*Honorary Secretary, Birnagar Palli Mandali.*

[December 17, 1931].

It will be remembered that Dr. P. Sur of the Government Malaria Research Laboratory at Krishnagar found sporozoites in *Anopheles philippinensis* caught in nature first in the villages adjoining the town of Krishnagar in 1927 and subsequently at Birnagar (Bengal) in 1928. Certain entomologists failed to find evidence of malarial infection in this species in Assam and one of them thought that Dr. Sur's work required confirmation by another working under similar conditions. The matter formed the subject of discussion in a previous paper, (Bose, 1931).

We have since started a malaria research laboratory at Birnagar not only for a confirmation of this point but also for a detailed study of the habits of *A. philippinensis*. With regard to the first point we carried out dissection of mosquitoes of this species only, caught in nature during the months September to November 1931. The results of the dissection are shown in the table below :—

	DISSECTION MADE BY				Percentage of infection.
	DR. S. C. BHATTACHARJI.		MR. S. DAS GUPTA.		
	Number dissected.	Number found infected.	Number dissected.	Number found infected.	
September (from 20th)	..	..	73	1	1·4
October .. ..	..	..	204	5	2·5
November .. ..	80	2	142	3	2·3

The distribution of these infected specimens in the four Wards of the Birnagar Municipality were as follows :—

Locality.		Number found infected. Sporozoites.	REMARKS.
Ward	I ..	6	All from one and the same house.*
"	II ..	3	
"	III ..	0	
"	IV ..	2	

\* Out of 13 persons living in the house, 8 suffered from fever and none of them took quinine regularly or in doses prescribed by the local medical officer.

It is a matter of great satisfaction that the infectivity of *A. philippinensis* has been corroborated by two of our workers. I am grateful to the Director, Malaria Survey of India, for the trouble taken in verifying the stained sporozoites in all the eleven slides sent to him at Kasauli for examination.

The habits of *A. philippinensis* are now being studied but in making collections from dwelling houses we have noticed this year also (1931) that it is more commonly found in these situations at night-time than during the day, as will be seen from the table below. The collection in this case was made by one and the same person who devoted equal hours, morning and night.

1931 September.	Night collection (8 to 11 p.m.).	Day collection (6 to 9 a.m.).
15th	11	2
16th	12	3
17th	22	5
18th	15	3
19th	13	2
21st	19	9
22nd	13	5
23rd	13	6
25th	17	2

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**SOME EXPERIMENTS TO DETERMINE THE EFFICACY OF  
WIRE GAUZE SCREENS OF DIFFERENT APERTURES  
IN EXCLUDING ANOPHELINE MOSQUITOES  
IN INDIA.**

BY

CAPTAIN H. W. MULLIGAN, M.D., I.M.S.,

AND

JEMADAR ABDUL MAJID, I.M.D.

*(Malaria Survey of India, Karnal, Punjab).*

[December 30, 1931].

IN considering the use of screening as an anti-malarial measure, it is obviously a matter of the utmost importance to utilize a type of wire gauze which will allow of the maximum amount of light and ventilation, and at the same time afford adequate protection against the malaria-carrying mosquitoes of the locality.

Covell (1931) has given an admirable summary of the present state of our knowledge of the use of screening, and has pointed out that many of the experiments already recorded omit the one essential point, viz., the actual size of the aperture in a given gauze screen. This author has made it abundantly clear that the number of openings to the linear inch is a totally inadequate measurement, since the size of the actual aperture will vary within wide limits, depending on the thickness of the wire used in manufacturing the screen. He emphasizes the urgency of acquiring fresh knowledge on this subject. The strength and durability of the screen are other points of considerable importance, but the first essential is to determine the largest aperture which may be employed with safety. At present there appear to be no recorded data regarding the optimum size of aperture which will exclude the malaria-carrying mosquitoes of India, and it is the purpose of the present paper to record some preliminary observations on this subject.

Working in a restricted locality it is obviously impossible to carry out experiments with all the malaria-carrying mosquitoes of India. *A. culicifacies* is one of the smallest, and at the same time one of most dangerous, malaria carriers in India. This small, compact mosquito is almost universal in its distribution in India. It may be taken that a gauze screen which will effectually exclude this species will also serve as an efficient protection against most of the other malaria carriers of India. *A. culicifacies* was therefore the species chosen with which to carry out the majority of the present experiments. Specimens of this species caught in nature, as well as specimens bred in the laboratory and kept for at least twenty-four hours after emergence, were utilized.

#### *Methods of investigation.*

Rectangular skeleton boxes were constructed in such a way that five sides could be fitted with interchangeable gauze panels, while the sixth (top) side was permanently closed with a sheet of cardboard having a small opening in the middle. A set of panels was prepared from each of the different types of gauze to be tested, so that in order to test a given gauze, it was necessary only to fit a skeleton box with panels made from gauze of that particular type.

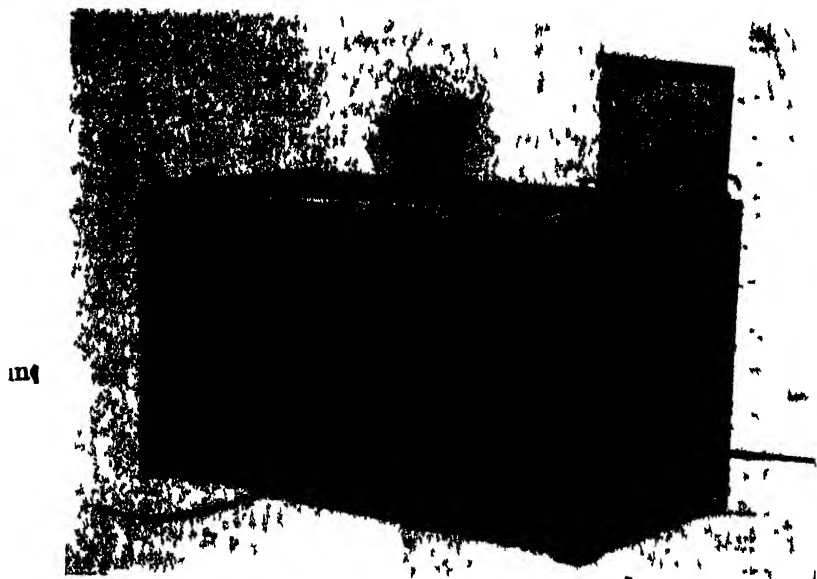


Fig. 1.

Fig. 1 shows one of the boxes used in these experiments. The capacity of the box is approximately 432 cubic inches.

In order to carry out an experiment a known number of mosquitoes was introduced into a gauze box through the opening in the cardboard lid which was then closed with a plug of cotton-wool. By means of hooks at the four top corners, the gauze box was then suspended in a large muslin breeding cage. A basin of water and some raisins were placed on the floor of the breeding cage in order to offer inducement to the mosquitoes to escape through the gauze. The mosquitoes were left alone and undisturbed except for regular inspections at 24, 48 and 72 hour intervals when the number of mosquitoes which had escaped was noted. At the end of an experiment the number of mosquitoes remaining in the gauze box was counted, this count including any mosquitoes which had died during the experiment. Several experiments were carried out with each type of gauze tested.

#### *The types of gauze tested.*

Panels for the skeleton boxes were prepared from seven different types of gauze, ranging from eleven to twenty openings to the linear inch. As has already been stated the number of apertures per linear inch is an inadequate measurement, and it was therefore necessary to ascertain, as accurately as possible, the actual size of the aperture in each sample of gauze. This was done by calculating the proportion of one linear inch of gauze which was not occupied by wire and dividing the result by the number of openings per linear inch. The Imperial Standard Wire Gauge was employed to measure the thickness of the wire in each sample of gauze. The estimations for each sample of gauze, which are tabulated in Table I are those given by the makers, Messrs. George Christie, Ltd., of Glasgow, and verified by us as described above. It should be noted that the size of the aperture is recorded as the actual breadth of one side of the aperture and does not refer to the area of the aperture.

#### *Results of the experiments.*

The numbers of *A. culicifacies* which passed through the different types of gauze tested are summarized in Table II. These figures represent the total number of mosquitoes which escaped during the course of several observations made with each type of gauze.

#### *Discussion of results.*

Before considering the actual results it is necessary to make some reference to the conditions under which the experiments were carried out. MacArthur (1923) has stated that 'we are not concerned with what mesh a frenzied mosquito imprisoned in a test tube may struggle through in order to escape from captivity, but with the mesh that a free mosquito will pass under natural conditions to obtain food'. There is no doubt, however, that mosquitoes in

nature will, at times, make most persistent effort to pass through gauze screens. One of us (H. W. M.) has repeatedly observed large numbers of mosquitoes making most determined, and sometimes successful, efforts to escape through screened doors and windows. In this case the inducement was apparently to gain a more favourable environment rather than to obtain food. In the tropics there is a very marked difference between the indoor and outdoor relative humidity in the morning and in the evening, and at these times mosquitoes can be observed to make most pertinacious attempts to escape from, or gain entrance to, a screened bungalow.

It is considered that the conditions obtaining in the present experiments, while admittedly artificial, give a fair indication of the capabilities of mosquitoes to pass through a given wire screen. It cannot be said that the mosquitoes were either unduly restricted, or in a condition which could be described as frenzied.

A glance at Table II will show that *A. culicifacies* is physically capable of passing through an aperture of 0.050 inch square. Only three of the 217 specimens of this species introduced into the box escaped within 72 hours. Using gauze with an aperture of 0.055 inch square 5 out of 176 specimens of the same species escaped within 72 hours, while as many as 18 out of 229 specimens (of the same species) escaped through gauze having an aperture of 0.060 inch square in 24 hours.

It would appear that, for practical purposes, screencloth having an aperture of 0.055 inch square would afford adequate protection against *A. culicifacies*. To render it physically impossible for this dangerous species to pass through it would be necessary to employ screencloth having an aperture of somewhere between 0.045 and 0.050 inch square, but it is not considered that the sacrifice of light and air incidental to the use of such a fine mesh would justify its employment. It would be better to risk the entry of an occasional stray mosquito.

An experiment was carried out with *A. listoni* using gauze with an aperture of 0.055 inch square. Although this is a very small mosquito, and a most pertinacious one, not a single specimen escaped through this aperture in 24 hours. A similar observation with *C. fatigans* gave an absolutely negative result.

It is interesting to note that the screencloth employed in proofing the barracks of British troops in India has an aperture of between 0.055 and 0.057 inch square. It would appear from our experiments that this is about the optimum aperture for general use in India or, at any rate, in the Punjab. The standard screencloth used by the Public Works Department, Punjab, has an aperture of 0.063 inch square, which while not absolutely proof against *A. culicifacies* under the conditions of our experiments, is considered to afford



considerable protection against this species and probably almost complete protection from most of the other mosquitoes of this province.

Le Prince and Orenstein (1916) working in Panama concluded that an aperture of 0.0485 inch square would allow the passage of *Stegomyia fasciata* only under conditions of stress, while an aperture of 0.046 inch square would exclude this species altogether. MacArthur (1923) expresses the opinion (based on experiments with *S. fasciata*) that screencloth having an aperture of 0.059 inch square should suffice to exclude mosquitoes from buildings. Earle (1930) working in Porto Rico found that an aperture of 0.053 inch square was sufficient to exclude practically all the mosquitoes he tried, and concluded that for most places an aperture of 0.056 inch square is sufficient.

#### *Further research.*

It is obviously impracticable, in a restricted locality, to carry out experiments of this nature with all the malaria-carrying mosquitoes of India. Should any worker in India desire to make further observations with the species of his own area we should be prepared to supply on loan the necessary equipment as described above. It would be particularly interesting and instructive to have results of experiments of this nature with such species as *A. minimus*, *A. aconitus*, etc. It is suggested that in dealing with laboratory bred specimens, the gauze cage might be suspended inside the investigator's mosquito net at night thus offering the captive mosquitoes the inducement of a human blood meal.

When climatic conditions are again favourable it is hoped to carry out further tests by utilizing two proofed rooms separated by a door with interchangeable gauze panels, the mosquitoes being placed in one room and a human volunteer in the other.

#### *Summary and conclusions.*

Experiments carried out with *A. culicifacies* to determine the largest aperture in gauze screens which may be used with safety show that, considering the advantages of light and air on the general health of a population, an aperture of 0.055 inch square is probably sufficient, under ordinary conditions, to exclude this species, and presumably therefore most of the other carriers in India. Such an aperture has been shown to prevent the passage of *A. listonii*, another small and dangerous species, and of *C. fatigans* the common Indian culicine. These findings compare closely with the results obtained by workers in other countries with different species. The gauze used for screening the barracks of British troops in India (0.055 to 0.057 inch square aperture) is probably about the optimum, while that used by the Public Works Department, Punjab (aperture 0.063 inch square), is probably effective against most species

in this province but will permit the passage of *A. culicifacies* in comparatively small numbers under experimental conditions.

#### ACKNOWLEDGMENTS.

We should like to express our indebtedness to Lieut.-Colonel J. A. Sinton, V.C., O.B.E., I.M.S., under whose direction this work was carried out, and to Messrs. George Christie, Ltd., Glasgow, who, at Colonel Sinton's request, kindly manufactured the screencloths used in these experiments to his specifications, and supplied them at cost price.

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TABLE I.

*Particulars relating to the different wire gauze screens used.*

Number of apertures per linear inch.	Diameter of wire in inches.	Proportion of one linear inch of gauze occupied by wire.	Proportion of one linear inch of gauze not occupied by wire.	Breadth of each aperture in linear inches	Clear ventilating space (per cent).
11	0·021	0·2310	0·7690	0·070	59·29
12	0·0183	0·2196	0·7804	0·065	60·84
13	0·0169	0·2197	0·7803	0·060	60·84
14	0·0164	0·2296	0·7704	0·065	59·29
15	0·0166	0·2490	0·7510	0·060	56·25
16	0·0175	0·2800	0·7200	0·045	51·84
20	0·0150	0·3000	0·7000	0·035	49·00

TABLE II.  
Summary of experiments performed to ascertain the capacity of *A. culicifacies* to pass through wire gauze screens having different apertures detailed in the table.

Number of apertures per linear inch.	Breadth of each aperture in inches.	Number of experiments performed.	NUMBER OF <i>A. culicifacies</i> PLACED IN CAGE.						NUMBER WHICH ESCAPED WITHIN 24 HOURS.						NUMBER WHICH ESCAPED WITHIN 48 HOURS.						NUMBER REMAINING IN CAGE AFTER 72 HOURS.						TOTAL NUMBER WHICH ESCAPED WITHIN 72 HOURS.					
			Bred			Wild			Bred			Wild			Bred			Wild			Bred			Wild			Bred			Wild		
			M	F		M	F		M	F		M	F		M	F		M	F		M	F		M	F		M	F		M	F	
11	0.070	6	44	49	14	123		2	16		11		1	3		7		41	30	14	105		3	19		3	19		18		..	
12	0.065	7	63	50	3	108		4	7		1		1	..		2		58	42	3	103		5	8		5	8		3		..	
13	0.060	7	39	75	20	75		3	11		..		4	2		..		56	62	20	71		3	13		3	13		4		..	
14	0.055	5	..	..	25	151		..	..		1	1	..	..		2		..	..	24	147		..	..		..	..		1	4		..
15	0.050	3	..	..	34	183		..	..		..	1	..	..		2		..	..	34	180		..	..		..	..		..	3		..
16	0.045	3	..	..	64	255		..	..		..	..	..	..		..		..	..	64	255		..	..		..	..		..	..	..	..
20	0.035	2	..	..	44	176		..	..		..	..	..	..		..		..	..	44	176		..	..		..	..		..	..	..	..

M means male *A. culicifacies*. F means female *A. culicifacies*.



## ULTRAVIOLET ABSORPTION SPECTRA STUDIES IN MALARIAL SERA.

BY

N. D. KEHAR, M.Sc.  
(*Malaria Survey of India, Kasauli*)

[April 8, 1932]

VARIOUS workers have shown that the blood of patients suffering from malaria differs from the normal in several of its chemical and physical properties, e.g., sugar, cholesterol, bilirubin, surface-tension, etc.

In view of these deviations from the normal it was decided to make a study of the ultraviolet absorption spectra of the sera from normal and malarial individuals. It was hoped that in this way the variations in the constituents of the blood in malaria would be reflected in the alteration of these spectra and that it might be possible, by studying spectra during the malarial rigor, to correlate changes observed therein with some of the physico-chemical observations previously investigated.

### *Technique*

The spectra were photographed with a Hilger Quartz Spectrograph. The source of illumination employed was an arc between two iron electrodes. Carbon, copper and manganese arcs were found unsatisfactory. Balv's absorption tube with quartz faces was placed on a level, between the source of illumination and the spectrograph. The absorptions were photographed through varying thicknesses of the serum dilution regulated by means of a plunger. Ilford's Special Rapid plates H and D 700 were used smeared with a light transformer oil which was found useful in increasing their sensitivity.

The subjects selected for these experiments were young adult Indians suffering from a fresh infection of malaria. The patients had no history of any attack of malaria during the previous three months and had not taken any quinine within two weeks of the date on which the blood was taken for examination. Special care was taken to see that the patients showed no signs of any other disease.

The blood was obtained by vein puncture. The serum was separated from the cells by centrifugalizing and only those sera which did not show any trace of hæmolytic were utilized. The serum was carefully pipetted off from the centrifuge tube and was diluted with physiological saline to make concentrations of 1:5, 1:10, 1:20, etc., up to 1:100. The spectra of various dilutions were tried and it was found that a dilution of 1:100 gave well-defined bands. The initial thickness of the serum-saline dilution in the Baly's tube was adjusted to give a range which showed the optimum development of the characteristic absorption bands.

Seven photographs of the spectra of both normal and malarial sera were taken on each plate. Each photograph represents a spectrum seen through different thicknesses of the serum-saline dilution within the range referred to i.e., 4-10 mm. in thickness increasing by one millimetre at each observation.

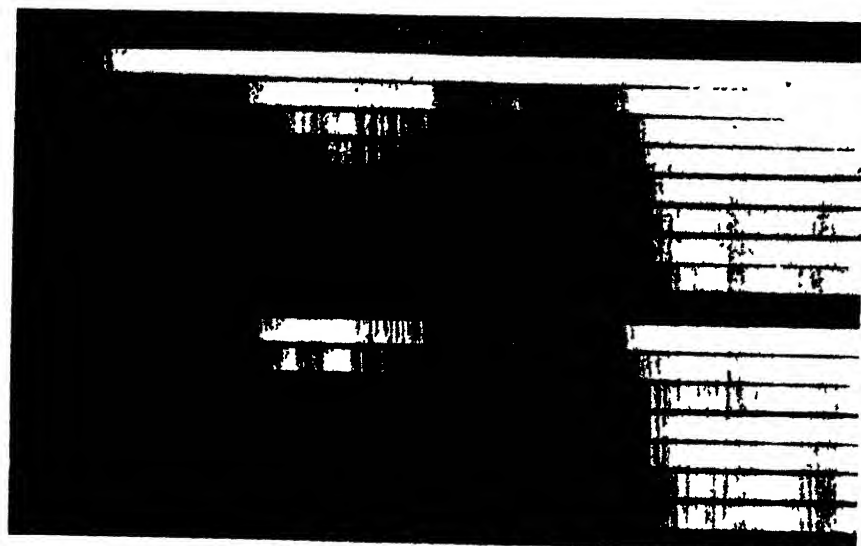
In this way sera from malarial and normal cases were investigated under identical conditions of dilution, thickness and exposure. The exposure given was 10 minutes in each case. The plates taken from the normal and malarial sera are, therefore, considered to be strictly comparable.

#### *Results and discussion.*

Ten specimens of normal and nine of malarial sera have been examined with results, which are practically constant. For convenience in comparing the normal and the malarial spectra photographs were taken on the same plate through varying thicknesses of liquid in the Baly's tube. The history of the cases is given below :—

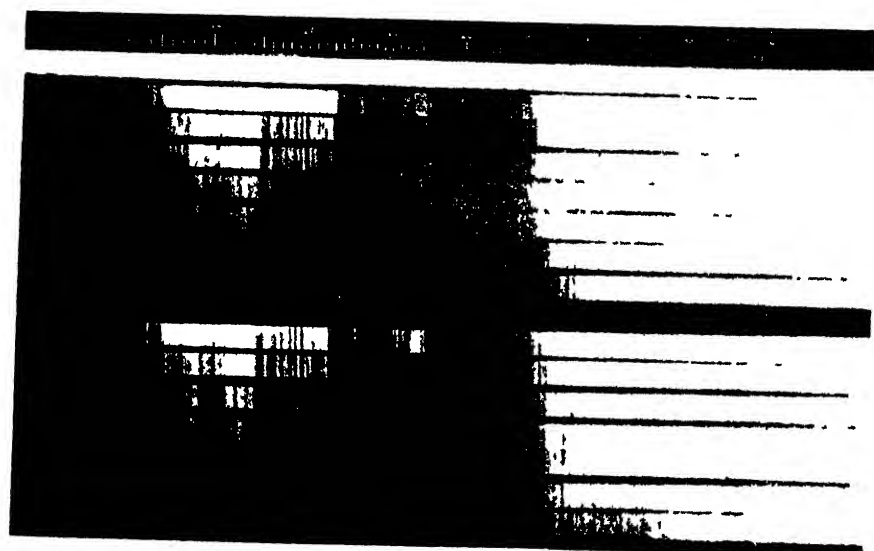
Case number.	Date.	Name.	Age.	Type of infection.	Time of taking blood.	Temperature.	REMARKS.
1	10-11-31	H. S.	35	<i>Plasmodium falciparum.</i>	2-30 p.m.	102°F.	Blood taken 4-7 hours after rice and milk diet.
2	12-11-31	N. M.	28	Do.	2 "	104°F.	"
3	13-11-31	J. S.	22	Do.	5 "	103°F.	"
4	18-11-31	Rah.	33	Do.	3 "	103°F.	"
5	24-11-31	Ahd.	23	Do.	2-30 "	104.5°F.	"
6	26-11-31	N. D.	25	Do.	3 "	103°F.	"
7	26-11-31	P. L.	36	Do.	3-30 "	104°F.	"
8	1-12-31	D. R.	30	Do.	3 "	103°F.	"
9	3-12-31	W. S.	27	Do.	4-50 "	102°F.	"

# PLATE VI.



Cu.  
A  
B  
C  
D  
E  
F  
G  
A'  
B'  
C'  
D'  
E'  
F'  
G'

Fig 1  
A—G Normal  
A'—G' Malarial



Cu.  
A  
B  
C  
D  
E  
F  
G  
A'  
B'  
C'  
D'  
E'  
F'  
G'

Fig 2  
A'—G' Normal  
A—G Malarial.  
(Blood serum spectra).





# PLATE VII



Fig. 3  
M and N indicate maternal and normal blood serum spectra

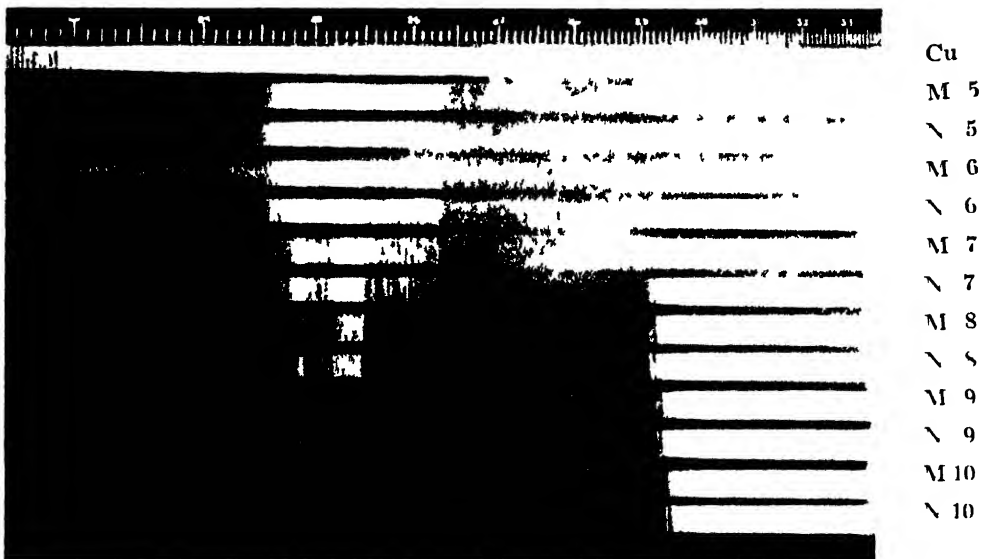


Fig. 4  
M = Maternal, N = Normal  
Figures represent thickness of serum saline dilution in mm



Before proceeding to record the spectra of sera, a copper spectrum was taken for the sake of comparing and distinguishing these lines from others. It was found that by putting the serum dilution in the Baly's tube, the spectra were affected a great deal. All the lines with copper, whose wave lengths were less than 2,400 Angström units, were cut off. Plate VI, figs. 1 and 2 show two of the actual spectrophotographs giving out clearly the difference between the normal and the malarial spectra. It is evident that whereas no selective absorption was found in the malarial sera, there is a general dimming of the spectra as compared to the normal.

For bringing out closer accuracy in comparison, the spectra of malarial and normal sera of the same thickness were taken side by side, photographs being normal and pathological alternatively, and it was found, as indicated by Plate VII, figs. 3 and 4, that whereas there are no new bands developed in the malarial sera the general dimness is characteristic. The results suggest that although no new constituents of malarial blood have been detected in the ultraviolet region, there is evidence of their varying in quantity as revealed by the spectra.

#### *Summary and conclusions.*

(1) The ultraviolet absorption spectra of normal and malarial sera are presented.

(2) The malarial sera do not show any selective absorption in this region of the spectrum as compared with the normal.

(3) In general, it appears that the absorption is greater in malarial than the normal serum throughout the spectrum studied. It will be interesting in this connection to examine the scattering power, which might give important information regarding the nature of the colloidal material in the malarial serum.

I am indebted to Dr. S. S. Bhatnagar, D.Sc., Director, University Chemical Laboratories, Lahore, for allowing me the use of the Quartz Spectrograph and to Lieut.-Col. J. A. Sinton, M.D., D.Sc., I.M.S., and Capt. H. W. Mulligan, M.D., I.M.S., for their keen interest and encouragement during the course of this research.

# HOYLE'S PURE PARIS GREEN

## *Specification :—*

$\text{As}_2\text{O}_3$	...	54—58 per cent.
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COLONEL SIR RONALD ROSS, K.C.B., K.C.M.G., F.R.S., LL.D., I.M.S. (retd.)  
(1857-1932).

*'Let us now praise famous men, for their work continueth,  
greater than their knowing'.—Kipling.*

WITH the death of Sir Ronald Ross on 16th September, 1932, India has lost one of the greatest of her sons. Ross, with Manson, Laveran and Koch, was one of that great group of pioneers who founded the modern science of tropical medicine, and he was the last survivor of those famous workers. The strides which have been made in tropical medicine, since Laveran discovered the malaria parasite in 1880, have probably been greater than in any other branch of medical science. The majority of this development has taken place since the epoch-making discovery by Ross in 1897 that the mosquito transmitted malaria.

Every inhabitant of the tropics, whether trader or missionary, soldier or sailor, engineer or doctor, fisherman or agriculturist, miner or explorer, mother or daughter, owes a debt of gratitude to this great scientist. Many individuals of unborn generations will owe their existence to his discovery.

If one considers the statement of that great physician Osler 'if a census were taken among the world's workers on disease, the judgment to be based upon the damage to health and direct mortality, the votes would be given to malaria as the greatest single destroyer of the human race', it is not too much to say that Ross's discovery was one of the greatest, probably the greatest, made in medicine in the last half century. It has saved innumerable lives and has made practicable the development of large areas of the tropics, which were previously uninhabitable. A striking tribute, among many, was paid to the value of his discovery by that famous tropical sanitarian, General Gorgas, who said 'It seems to me not extreme, therefore, to say that it was your discovery, that enabled us to build the Canal at the Isthmus of Panama'.

The benefits which India, with her great incidence of malaria, reaps and will reap from Ross's great discovery, should always keep his memory green in this country. A Gate of Commemoration was opened in 1927 by Lord Lytton, the Governor of Bengal, at the Presidency General Hospital, Calcutta. The doors of the gate bear a bronze medallion with Ross's effigy and the inscription—'In the small laboratory 70 yards to the south-east of this gate, Surgeon-Major Ronald Ross, I.M.S., in 1898 discovered the manner in which malaria was conveyed by mosquitoes'. The Ross Field Experimental Station for Malaria at Karnal, Punjab, was instituted in 1926 by the Indian Research Fund Association to continue those researches in malariology with which Ross's name will always be associated. This institution contains the permanent field and teaching laboratories of the Malaria Survey of India.

India can proudly claim Sir Ronald Ross especially as her own, for he was born at Almora in the Kumaon Hills, on 13th May, 1857, of a family with many associations with this country. He spent 18 years as an Officer of the Indian Medical Service and it was in India that he made his famous discovery.

Sir Ronald Ross received his medical training at St. Bartholomew's Hospital and he entered the Indian Medical Service in 1881. His early inclinations were towards literature, music and mathematics, and it was only after several years' service in India that he turned his mind seriously towards medical research. During leave in England in 1888, he obtained the newly instituted Diploma of Public Health and took a course of bacteriology with Professor Klein. These studies were the foundation of his expert knowledge of microscopy, which proved so useful to him in his later work.

As the result of his observations in India at this time, he wrote an essay on malaria for which he was awarded the Parke's Memorial Prize on his return to England in 1895. In this thesis he put forward evidence against the generally accepted theory of the period, that malarial infection was caused by miasma arising from swampy areas. As a result of this work he came in contact with Sir Patrick Manson, who demonstrated to him Laveran's malaria parasite, about which he, as well as many other workers, still maintained a considerable degree of scepticism.

He returned to India filled with enthusiasm for further research work on malaria. His chief object was the investigation of the theory that mosquitoes were responsible, in some manner, for the spread of malaria, a hypothesis which had been suggested in 1883-84 by King, Laveran and Koch, and of which Manson was a strong supporter.

It is very difficult nowadays to appreciate the magnitude of the task which confronted Ross when he returned to India in 1895 and rejoined his regiment at Secunderabad. At that time scientific medicine, as known at present, was in its infancy and medical protozoology or entomology had hardly been born. Except for the discovery by Kilborne and Smith in 1893, that bovine piropasmosis was transmitted by ticks, no one had dreamt of the spread of protozoal diseases by the bites of arthropods. At this time only 4 species of mosquito had been recorded from India, while now nearly 300 are known, and Ross had no entomological training and no literature to guide him when he set out to explore the uncharted seas of the transmission of disease by these insects.

Ross started to test Manson's tentative suggestion that the disease might be transmitted by drinking water contaminated by mosquitoes which had fed on malarial patients, but his experiments met with no success.

At this time the nature of the gametocytes of the malaria parasite was unknown and the flagellum was believed by some workers to be an independent organism and by others a degeneration product produced during the dying stages of the parasite. Ross collected innumerable mosquitoes and after feeding them on malarial patients dissected them. His first discovery was that exflagellation of the male gametocyte could take place in the stomach of a mosquito fed on a suitable case. He continued his investigations, stimulated by Manson's enthusiastic support and his dictum — 'Follow the flagella.'

The chase was a long one. Through 2½ years of unceasing toil discouraged by heart-rending disappointments and technical difficulties and hampered by cholera epidemics and the exigencies of military service, with indomitable perseverance he struggled on, sustained by the counsel and sympathy of Sir Patrick Manson. About noon on 20th August 1897, in the heat of an Indian summer, he saw for the first time the developmental stages of the human malaria parasite in the stomach wall of an Anopheline mosquito and thus opened up a new era in the history of disease transmission.

At this time military duties interrupted his work and not until the following year when he was placed on special duty at Calcutta had he an opportunity of completing his research. In July 1898 he was able to telegraph to his friend, supporter and confidant, Sir Patrick Manson, that he had proved the mosquito cycle of the malaria parasite by his experimentation with bird malaria and the Culex mosquito. In the years which have elapsed since he made his discovery under such trying conditions very little of outstanding importance has been added to his original description.

The details of the mosquito-malaria cycle are so extraordinary that they must have sounded like a fairy tale to the scientific world of the day, yet they were so convincing that when Manson announced the discovery at the Annual Meeting of the British Medical Association in Edinburgh in July 1898 the members rose and cheered. Ross's results were quickly confirmed, and the enormous value and scientific importance of his discovery recognized.

Although Ross sometimes spoke of the luck which attended his discovery, one cannot but think that a lesser man not possessing his grit, energy, determination and ability, would have given up in despair before the enormous difficulties which beset his path.

Sir Ronald Ross left India in 1899 and was quickly appointed to a teaching post at the newly-formed School of Tropical Medicine in Liverpool. While in this post he took part in many expeditions to the tropics in connection with malarial investigations and prevention. The

chief of these were to the West Coast of Africa, to Ismailia, to Greece and to Mauritius.

There is no branch of malariology which has not felt his influence. During the 13 years in which he was at Liverpool he taught and inspired many workers, not only in connection with malaria, but also in relation to many other tropical diseases.

At the outbreak of the Great War, he acted as Consultant in Tropical Diseases to the Army, with the rank of Colonel. He visited Egypt and Salonika in this capacity and was on a ship which was torpedoed. After the War he was appointed Honorary Consultant in Tropical Diseases to the Ministry of Pensions. He was for many years a member of the Colonial Office Advisory Committee and of the Advisory Board of the Indian Research Fund Association.

Some years later a public subscription was started which resulted in the foundation of the Ross Institute for Tropical Diseases at Putney Heath, London. This institution was inaugurated by H. R. H. The Prince of Wales on 15th July, 1926, with Sir Ronald Ross as Director-in-Chief.

Unfortunately he had a paralytic seizure in 1927, but even this did not stop his unbounded energy and he continued at work, more especially in his mathematical studies, until a few months before his death.

Ross's genius manifested itself in many forms. Although he will always be remembered for his medical researches, yet his work on pure mathematics and pathometry, more especially in relation to malaria, was of a high order. His scientific achievements have won for him world-wide fame and have overshadowed his work in the field of art. He was not only a musician of no mean ability but also a novelist and a poet. Indeed at one time he seriously contemplated abandoning medicine for literature. Of his poetry, Mr. John Masefield, the Poet-laureate, has spoken in most eulogistic terms.

Sir Ronald was always a staunch friend and an enthusiastic helper to research workers who consulted him. The campaign for improving the emoluments, and for the state endowment of research workers received his strong support.

Sir Ronald Ross was made F.R.S. and F.R.C.S. in 1901. He was decorated C.B. in 1902, K.C.B. in 1911 and K.C.M.G. in 1918. His great discovery gained for him the Nobel Prize for Medicine in 1902 and very many Governments and Universities awarded to him honours and degrees in recognition of the great benefits which his discovery has conferred upon humanity.

All over the world, workers in tropical medicine, and more especially in malariology, will deplore the loss of a great scientist and his old pupils will also mourn the passing of a valued friend and teacher.



## THE ANOPHELINE MOSQUITOES OF HYDERABAD, DECCAN, AND THEIR CONTROL.\*

BY

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[February 22, 1932.]

ALTHOUGH a number of observations upon the anopheline fauna of Hyderabad City have been made during the last 21 months, our knowledge of this subject is far from complete. The only previous records concerning anopheline mosquitoes and malaria in this area which are available are :—

(1) Hehir (1927) refers to the mild endemicity of malaria in certain areas (Hussain Sagar) in Hyderabad.

(2) James and Liston (1911) mention a few species of anopheline mosquitoes recorded from Secunderabad and Aurangabad.

(3) Watson (1928) has written a note on 'Malaria in Hyderabad'.

It appears from the literature available that no systematic or comprehensive survey has been undertaken either of the incidence of malaria or of the anopheline fauna of Hyderabad City.

A department consisting of one Civil Surgeon, one Assistant Surgeon, four Sub-Assistant Surgeons, four Sanitary Sub-Inspectors and forty labourers was organized by Col. J. Norman Walker, Director, Medical and Sanitary Departments, to undertake the eradication of malaria in Hyderabad City. The results achieved have been very successful.

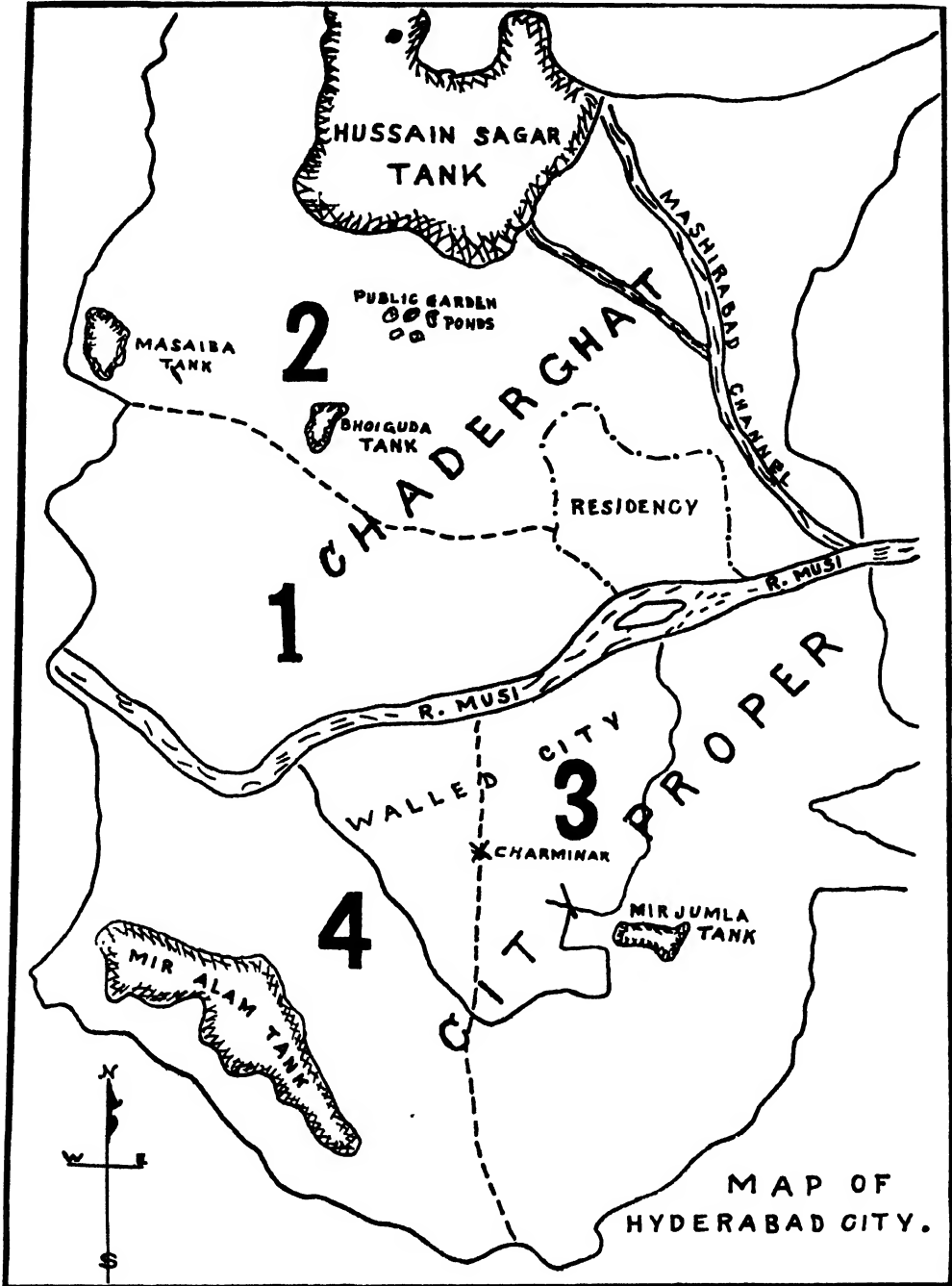
A systematic survey was started in January 1930, and the present article embodies the results of observations carried out both in the field and in the laboratory.

### TOPOGRAPHY.

The City of Hyderabad, the capital of the Nizam's Dominions, covers an area of 33.28 square miles, and is situated 1,719 feet above sea level. It lies

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\* Essay awarded the prize medal in the Annual Prize Competition of the Osmania Medical College Old Students' Association.



*Note.*—The numerals refer to the sections of the city.

between 17° 15" latitude and 78° 30" longitude. The average mean annual temperature is 91°F. The maximum temperature usually rises to 108° or 110°F. in May, and the minimum falls to 61°F in December. The average rainfall is 29 inches. The average relative humidity is 68 per cent, reaching as high as 79 per cent in July, August and September, and falling as low as 48 per cent in April and May.

The population of the city according to the 1931 census is 400,397.

There are large areas of rice cultivation within the city boundaries, amidst human habitations, viz., at Mui Jumla, Dubirpura and Chaderghat. The main source of water supply is the Gundipetti Tank, situated 12 miles from the city.

The River Musi, a tributary of the Krishna, runs through the City, dividing it into two almost equal parts. The area north of the river is called Chaderghat, and that to the south the City Proper. Although the bed of the river is over 600 feet wide, the main stream is not more than 50 to 60 feet in width. The sullage and sewage water of the City are discharged into the river at the New Bridge. Beyond Chaderghat Bridge and the Old Bridge (Puranapul) wet cultivation is carried on along the river banks. The excavation of sand is allowed in the bed of the river, and on account of this innumerable pools and pits are formed. Throughout its length, from the Old Bridge to Amberpet, the river is densely covered with water hyacinth.

CHADERGHAT is the area situated north of the River Musi. This area is less congested than the City Proper, and contains many buildings constructed on modern hygienic principles. As it is the healthiest part of the City, many of the nobles and officials reside here. Although it is almost surrounded by water, it is not important from a malarial point of view, as will be shown later. This area contains the following breeding places —

(1) *Hussain Sagar Tank* — This, the largest tank in Hyderabad, lies towards the north, and is the main source of water supply for Secunderabad. The eastern portion of the tank has a masonry bund, whereas the south-west, north and north-east portions are covered with water weeds and harbour larvæ. The seepage water from the tank on the western and eastern sides gives rise to many pools and ponds. There are also large marshy areas on the south and south-west aspects of the tank, due to seepage water.

(2) *The Mashrabad Channel* — This channel originates from Hussain Sagar Tank, and pursuing a winding course through wild vegetation and jungle, enters the River Musi at Amberpet. The area on both sides of this channel is very marshy, owing to seepages from Hussain Sagar Tank. The villages situated close by, which were once very prosperous and well populated, have been deserted on account of severe malaria. Wet cultivation is carried on along the banks of the channel.

(3) *Afzul Sagar or Bohiguda and Masahiba Tanks* — These are small tanks in which harmless species of anophelines breed.

(4) *Public Garden Ponds* — These are situated in a garden which is a place of recreation for the public of Hyderabad. They are ornamental ponds,

covered with lotus flowers and leaves, and form ideal breeding places for mosquitoes. Swans, ducks and other aquatic birds are present in the ponds.

(5) *Wells*.—There are about 1,000 wells in this area, most of which are used for the watering of gardens. Harmless species of mosquitoes breed in them.

The CITY PROPER includes the area south of the River Musi, and represents the ancient city. It is thickly populated, and the general sanitation is not satisfactory; in it is included the walled city. This area is especially noteworthy on account of the intensity of malaria prevailing in it, due to the presence of a large number of wells, in which dangerous species of mosquitoes breed. The breeding places in this area are :—

(1) *Mir Alum Tank*.—This was once the chief source of water supply for Hyderabad City, and is situated in the south-west quarter. The north-east portion of the tank, facing the City, requires anti-larval treatment.

(2) *Mir Jumla Tank*.—This lies in the centre of a congested locality, and is no longer a tank, being represented by a small drain and some swampy tracts. Most of the water is drained off from the head of the tank for wet cultivation.

(3) *Wells*.—It was not until our survey had been in progress for three months that we became aware of the presence of more than 6,000 open wells in this area. Many of the wells were found to be in private houses, and the openings of these are not more than  $1\frac{1}{2}$  feet in diameter. Practically every well forms a breeding place for *A. stephensi*, which is incriminated as the chief carrier of malaria in Hyderabad. The wells are used for washing and cleaning purposes, and in areas where the piped water supply is limited, well water is also used for drinking.

Besides the above-mentioned water collections, the other permanent breeding places are cisterns, fountains, pools and ponds.

Temporary breeding places are afforded by collections of rain water in borrow-pits and other excavations, and in utensils such as earthenware toddy-pots which are thrown away in the fields and elsewhere. In the course of the investigation many earthenware toddy-pots were found to contain innumerable larvæ, including those of several dangerous species, e.g., *A. stephensi*, *A. minimus*, *A. listonii* and *A. culicifacies*.

#### ANOPHELINE MOSQUITOES AND THEIR BREEDING PLACES.

The anophelines so far identified by me in Hyderabad City are as follows :—

1. *A. subpictus*.—A harmless species, which has not been proved to be a carrier of malaria in nature in India. It breeds in any kind of water, and is widely distributed throughout the City.

2. *A. stephensi*.—A dangerous natural malaria carrier, notorious as a well breeder. In Hyderabad it has only been found breeding in the City Proper, where its larvæ have been found in enormous numbers, especially in

the wells in the walled city. Some wells in No. 1 section and a few in No. 2 section were also found to contain larvæ of this species.

3. *A. fuliginosus* and *A. pallidus* breed abundantly in Hussain Sagar and Mir Alum tanks. Neither of these species is considered to be a dangerous malaria carrier in India, although the former has been suspected to transmit the disease in Bengal. It is not thought to play any rôle of importance in the spread of malaria in Hyderabad.

4. *A. minimus* and *A. listoni*.—These species have been found breeding in abundance in the Mashirabad Channel, Chaderghat area. Though they are potent carriers of malaria in other parts of India, they have not yet been found infected in Hyderabad.

5. *A. aconitus* has been found breeding in large numbers in the Mashirabad Channel and the River Musi, in the north-east and central parts of the City. This species has been found to carry malaria in nature in the Dutch East Indies, and although I have not yet found any specimens infected with malaria parasites, I have reasons to believe, as will be shown later, that this mosquito plays a part in the spread of malaria in this area.

6. *A. culicifacies* has been found breeding in the River Musi and Mashirabad Channel, and also in rain-water pools and wells in the City Proper. This mosquito is a dangerous natural carrier of malaria, and plays an important rôle in the transmission of the disease in many parts of India. It is probable that it will eventually be proved to be a carrier of malaria in Hyderabad.

7. *A. hyrcanus* and *A. barbirostris*.—These breed in enormous numbers in the Mashirabad Channel, and in swampy tracts formed in the rains. It seems to me that they contribute very little to malarial incidence in Hyderabad.

8. *A. vagus* is not abundantly found, and is not a carrier in nature.

9. *A. tessellatus*.—This species was first discovered by me towards the end of September 1931. A few larvæ were detected in a shady pool in a field outside the boundary wall of Yacuthpura, during an investigation as to the cause of the high intensity of malaria in that locality. It is of interest to note that no less than 10 species of anopheline larvæ were found in this area. We have as yet no adequate knowledge about *A. tessellatus* in Hyderabad.

The common breeding places of the anophelines found in Hyderabad City are summarized in tabular form with reference to those recorded elsewhere in India in Table I. Certain unusual breeding places of these species are also mentioned.

It must be borne in mind, when the question of the control of these species is discussed, that although some species show a special predilection for some particular breeding places, they are also capable of adjusting themselves to altered circumstances. To quote an example, *A. stephensi* is essentially a well breeder, but it is capable of breeding in rain-water pools, etc. Hence the malariologist should not be satisfied with merely proposing the permanent closing of wells with concrete domes or covers, but should persist in his

endeavours to detect the presence of these larvæ in every water collection, in addition to wells and cisterns.

During the last 21 months I have had the opportunity of examining at the laboratory more than 15,500 larvæ, which were collected from almost every type of breeding place. The prevalence and seasonal distribution of the various species are shown in Table II. Although there is yet much to be studied concerning the bionomics of these mosquitoes with regard to their seasonal prevalence, period of hibernation and æstivation, range of flight, etc., yet the observations made by me during the short period of the investigation may serve as a guide and form a good starting point for an exhaustive study of this subject in the future.

#### SEASONAL INCIDENCE OF ANOPHELINES.

The following observations are recorded in Table II :—

1. *A. hyrcanus* and *A. barbirostris* appear towards the end of September, increase in numbers in October, November, December and January, and disappear in February.

2. *A. culicifacies* appears towards the middle of June, is common during the months of July, August, September and October, and disappears in the latter weeks of November.

3. *A. aconitus*, *A. minimus* and *A. listonii* appear towards the end of November, breed in large numbers in January, February, March and April, and disappear towards the middle of May.

4. *A. stephensi*, though it breeds throughout the year, seems to be most active during the months of June, July, August and September.

It must be admitted that an extensive catch of adult mosquitoes could not be undertaken owing to the limited staff of workers available; nevertheless, Table III has been drawn up from the data collected, showing the number of adult mosquitoes caught, dissected and found infected with malaria parasites in Hyderabad City. So far, only one species, *A. stephensi*, has been found infected with malaria parasites, the percentage of infection being 3·5. A close study of this table with regard to the period of activity of the different species of mosquitoes will support the conclusions drawn from Table II.

#### INCIDENCE OF MALARIA.

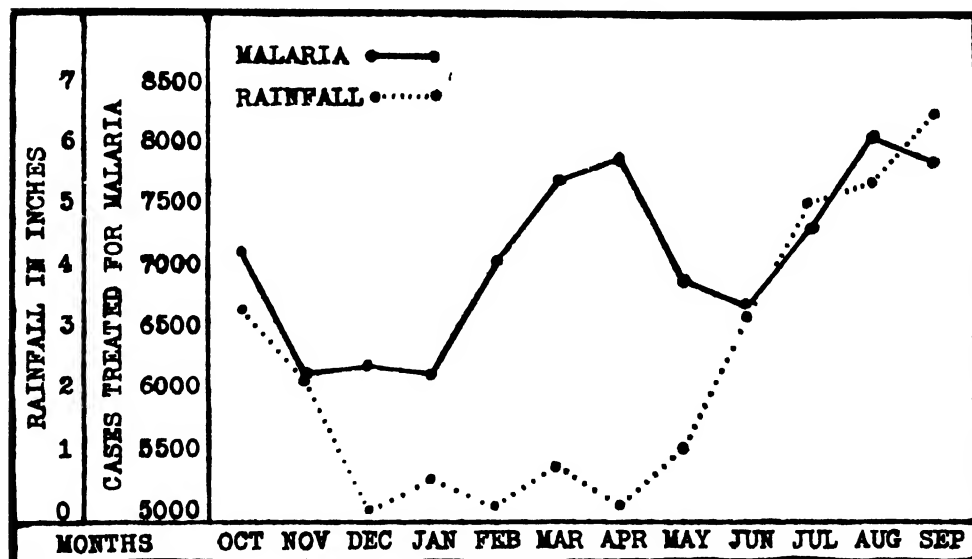
From a study of the Map it will be seen that although Chaderghat, the area north of the River Musi, is abundantly supplied with water and contains extensive breeding places like Hussain Sagar and Mashirabad Channel, yet the Civil Hospital returns of fevers treated as malaria and the splenic index only show a moderate degree of malarial endemicity in this area, i.e., the spleen rate is only between 10 and 25 per cent. But the area south of the River Musi, especially the walled city, which has a diameter of one mile with Charminar as the centre of the circle, shows a very high incidence of malaria, the spleen rate being over 50 per cent. The intensity of malaria in this area is due to

the large number of wells, which are situated in practically every house, and in which breeds the dangerous carrier, *A. stephensi*. Poorly built houses and overcrowding in this area are other conditions which afford shelter to mosquitoes, and predispose to the easy spread of the infection.

#### RAINFALL AND MALARIA.

Graphs 1 and 2 illustrate the relationship between rainfall and malaria. The graphs indicate that there are two malaria seasons during the year in Hyderabad, viz., the first season extending from February to May, and the second from July to October. The second malaria season bears a direct relation to the rainfall. With the increase of the rainfall there is a definite increase in fever cases also.

GRAPH I.

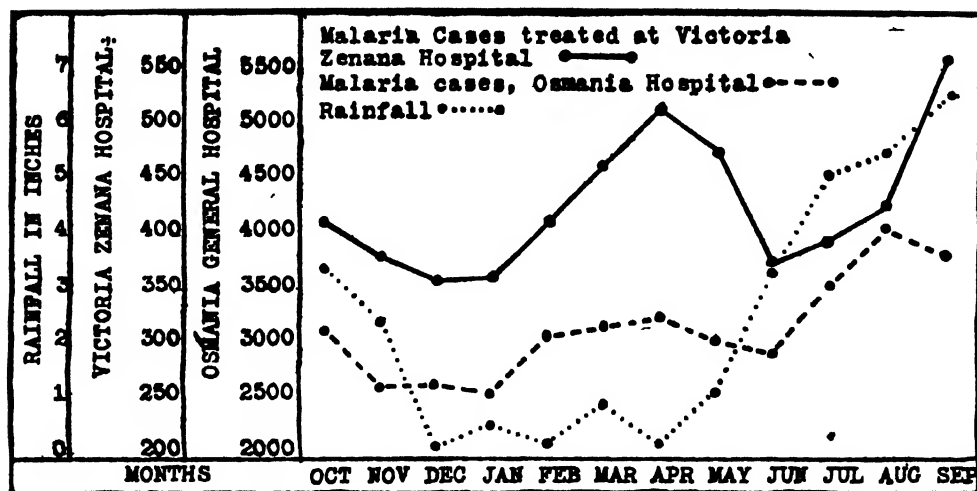


Relation between rainfall and malaria, compiled from the returns of five hospitals in Hyderabad City, 1923-1928

#### SPECIES OF ANOPHELINES RESPONSIBLE FOR THE TWO MALARIA SEASONS.

As has been shown, *A. stephensi* is responsible for a large amount of malaria in Hyderabad, and, deducing from observations recorded in Tables I and II, I am led to attribute the increase of malaria in the first season to *A. aconitus* and in the second to *A. culicifacies*. It is hoped that in the revised scheme, which provides for a wider scope for the investigation of this problem, the conclusions so far arrived at by me will receive confirmation

GRAPH II.



Relation between rainfall and malaria, compiled from the returns of Victoria Zenana Hospital and Osmania General Hospital, 1923-1928.

#### THE CONTROL OF ANOPHELES.

When undertaking any method of control involving large expenditure, it is essential for the local conditions to be studied in detail. The problem of malaria control differs in different places, and there is no single method that can be advocated for all conditions and all countries. During recent years there has been considerable controversy on the subject of control among men of outstanding reputation in the malaria world. Two schools of thought have arisen, viz., the 'modern school' and the 'old school'. Those representing the former hold that malaria is a social disease, i.e., diet, housing, economic status, general sanitation and other factors play a great part. They also maintain that anti-mosquito measures are impracticable on account of financial and administrative reasons, especially in wide areas with a scattered population. The 'old school', which has many supporters, holds that malaria is not a social disease, and that diet, housing, economic status and general sanitation play a negligible part in its spread. Sir Ronald Ross (1930), commenting on the controversy, makes the following remark: 'There is only one school of competent malariologists—those who do the work..... Many people seem to think that malaria control is responsible for expenditure; they forget that the disease itself costs much more money..... If, of course, malaria could be controlled by cheaper methods, one would be only too glad, but I doubt whether it can be done'.

In this City we have adopted anti-larval measures. The larvicides used are paris green in a one per cent dilution with screened road dust, and oil



mixture (brown kerosene oil 9 parts, crude oil one part, castor oil one per cent).

There are three methods of control :—

(1) Mosquito control, i.e., measures adopted to eradicate anopheline and culicine mosquitoes.

(2) Anopheline control, i.e., eradication of all anopheline mosquitoes.

(3) Species control, i.e., eradication of only those species of anopheline mosquitoes which have been incriminated as carriers of malaria.

The present-day malariologist does not usually endeavour to eradicate all mosquitoes in a malarious district. His measures are entirely concentrated upon the last method, species control. This method is not only more scientific, but it involves less expenditure. It is therefore within the reach of anybody who wishes to undertake an anti-malaria campaign.

Since a Malaria Department has now been organized in Hyderabad City for the first time, certain obstacles have been placed in our way by the public. We were therefore forced to adopt the method of general mosquito control, in order to impress the public with the value of the organization by demonstrating to them a marked reduction in the mosquito population. In spite of a vigorous campaign, a few complaints of the mosquito nuisance were still received, and in all these cases culicine mosquitoes were found to be the culprits. That the anopheline mosquito population was controlled to a large extent by our methods is shown by a marked diminution in the number of attendances for fevers treated as malaria at the Government Civil Hospitals, Hyderabad City, in spite of an increased total attendance for all diseases at these hospitals.

#### SOME LOCAL PROBLEMS REQUIRING SPECIAL ATTENTION WITH REGARD TO CONTROL MEASURES.

In addition to the usual difficulties which stand in the way of Public Health workers in India, viz., the ignorance and religious scruples of the people, the special problems connected with the control of malaria in Hyderabad City are :—

A. The River Musi, on account of (a) excavation of sand from the river bed, which gives rise to innumerable pits and pools and thus creates breeding places, and (b) profuse growth of water hyacinth.

B. The Mashirabad Channel and the marshy area around it.

C. The wet cultivation (rice) which is carried on amidst human habitations.

#### RECOMMENDATIONS.

The Malaria Department have made the following recommendations to Government :—

1. All wells in the City should be permanently closed, either by covering them with cement concrete, or by filling in.

2. The bed of the River Musi should be improved by canalization, or, preferably, by holding up the stream by a series of weirs (Ashford's falling shutters), so that the river may remain full and attain a considerable depth of water. Flushing should be done periodically, once a week if possible, since there is a danger that pools left in the bed of the river may become sources of mosquito breeding if it is done at longer intervals than this.

3. Water hyacinth should be removed and its growth prevented. As this water plant is considered to be a pest in many parts of India, a note on this subject is given as an Appendix to this paper.

4. The excavation of sand from the bed of the river should be prohibited, in order to prevent the formation of new pools.

5. The drainage scheme should be completed and connected up for the carriage of all sewage, sullage and storm water to the sewage farm outside the City.

6. Wet cultivation at Mir Junla, Dubirpura, etc., should be prohibited.

7. The following recommendations were made by Sir Malcolm Watson (1928) for the Mashirabad Channel and the marshy area below Hussain Sagar Tank :—

(a) To construct a concrete channel for the main stream of the Mashirabad Channel from Hussain Sagar Tank to the River Musi at Amberpet, and subsoil drainage for the seepages by means of properly made and laid out pipes.

(b) To drain and reclaim the land below Hussain Sagar Tank, and convert this semi-marshy area into public parks and play-grounds.

Sir Malcolm Watson's recommendations undoubtedly involve a large expenditure. The estimate for (a) alone amounts to nearly one lakh of rupees. I do not wish to comment on Sir Malcolm Watson's suggestions, for it will take time before the local problem can be thoroughly studied, and until this is done the merits and demerits of his recommendations cannot be weighed.

Paris green has had an extensive trial in our hands, and has proved very efficacious in the control of anopheline larvæ. Experiments in the field and laboratory were carried out by me on the same lines as those described by Sur and Sarkar (1929) of the Bengal Public Health Department, and the results obtained confirmed their findings in most respects.

The natural enemies of mosquito larvæ which help in our control in Hyderabad are *Dytiscus* and other water-beetles, and the fishes belonging to the families Cyprinidæ (*Nuria danrica*, *Barbus* sp., *Danio* sp.), and Anabantidæ (*Anabas scandens*).

My most grateful thanks are due to Col. J. Norman Walker, Director, Medical and Sanitation Department, and Dr. L. D. Khatri, Malaria Officer, for very kindly affording me facilities in my work. I am also thankful to the staff attached to the Malaria Department. I also wish to express my indebtedness to the Director, Malaria Survey of India, for verifying my results, and to Capt. P. J. Barraud, F.E.S., F.Z.S., Entomologist, Malaria Survey of India, for his ready help and advice to me whenever I was in such need.

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## APPENDIX I.

Water hyacinth (*Eichornia crassipes*), popularly known as 'pisachi tamarai' in Tamil and 'rakasi tamarai' in Telegou, belongs to the family Pontederaceæ. It is a native of Brazil, South America, and has now become a troublesome weed in other countries, notably Florida, Java, Australia and India. On account of its beautiful mauve flowers it has found its way into gardens in different countries. In Florida 'Admirers of this plant placed plants in the St. John's River in front of their houses to beautify their surroundings'. Water hyacinth first came to be seriously considered as a pest in Florida in 1890, in Queensland in 1895, in Cochin China in 1908, in Burma in 1913, and in Bengal in 1914. It can propagate itself both by seeds and suckers; but the plant spreads rapidly especially by means of small lateral branches, which occur in great numbers, and from all of which new plants can spring. When and how this plant made its home in the River Musi in Hyderabad is not known; probably from Madras Presidency, for it is found in Madras and in Vizagapatam District. Viosca (1925) in America advocated the use of water hyacinth in the control of mosquito larvæ, with the idea that it would act by covering the water so thickly as to prevent the respiration of larvæ. Barber and Hayne (1925) came to the conclusion that it was of little value.

This plant is a troublesome weed in Hyderabad. Besides giving shelter to adult mosquitoes and larvæ, the large, bladder-like leaf stalk and the dense growth of the plant mechanically interfere with the action of larvicides. In one instance, I found, in a portion of the River Musi near the New Bridge, densely covered with water hyacinth, large numbers of the larvæ of *A. aconitus*, *A. hyrcanus*, *A. fuliginosus* and *A. barbirostris*, breeding one yard away from the edge of the stream. Removal of the plant by hand is the method that is adopted. Recently during the rains we found that the flushing of the river had a good effect in washing away the plant, so that the river between the Old Bridge and Chaderghat Bridge is free from it.

The plant is useful for many purposes, viz., extraction of potassium chloride, manufacture of paper, preparation of ink from the flowers, utilization for fuel, etc.; but the chief uses for it are as fodder for cattle, and as manure on account of its richness in potash. If the people, particularly the agriculturalists, are made to utilize this plant as manure and as fodder for cattle, they will not only relieve the Malaria Department of labour and expense, but will also help in the efficient control of larval production.

## APPENDIX II.

TABLE I.

*Table of breeding places found in Hyderabad and their peculiarities.*

Species.	Commonest breeding places according to other observers.	Commonest breeding places in Hyderabad City.	Unusual breeding places.
<i>A. subpictus</i> ..	Found breeding in all kinds of collection of water.	Breeds anywhere and everywhere.	....
<i>A. stephensi</i> ..	Well breeder. Also in pools in river beds and by the side of streams.	Wells and cisterns and not found in pools in river beds.	Rain-water pools.
<i>A. culicifacies</i> ..	Slow running streams, irrigation channels, pools in river beds, and found in clean water and rain water.	River, channel and rain-water pools.	Tank, cistern (1), mudpot (1), wells (5).
<i>A. fuliginosus</i> ..	Swamps, small pools, of muddy water, in the bed of small streams, rice fields.	Tanks, channel, ponds and swamps.	Main stream of river (2), cistern (1), wells (5).
<i>A. pallidus</i> ..	In pools, in bed of small streams, tanks with vegetation.	Swamps and ponds.	Ci-tern (1).
<i>A. hyrcanus</i> ..	Rice fields, swamps, collection of (rain, canal, or river) water with vegetation along edges.	Channel, swamps, river covered with hyacinth, ponds.	Cistern (2), wells (5).
<i>A. barbirostris</i> ..	Shady pools along bed of slow running streams, ditches, and swamps.	Channel, swamps and ponds.	Wells (6).
<i>A. minimus</i> and <i>A. listoni</i> .	In wells, and according to Iyengar in stagnant fresh water in ponds and ditches.	Channel and river.	Wells (4), mud-pot (1), tank (1).
<i>A. aconitus</i> ..	Swift running irrigation channels, swamps.	Channel and river covered with hyacinth.	Wells (2).
<i>A. vagus</i> ..	Same as <i>subpictus</i> .	River, not commonly found	....
<i>A. tessellatus</i> ..	Disguised wells, swampy places with high grass and, according to Hacker, found in pools in dense jungle.	Partly shady pool in a field, found only once.	....

Figures indicate number of times found.

TABLE II.

Showing the number of anopheline larvæ identified and their seasonal prevalence from January 1930 to September 1931.

Months.	TOTAL.		<i>A. subpic-tus.</i>		<i>A. stephensi.</i>		<i>A. culici-facies.</i>		<i>A. fuligi-nosus.</i>		<i>A. palli-dus.</i>	
	Years.		Years.		Years.		Years.		Years.		Years.	
	1930	1931	1930	1931	1930	1931	1930	1931	1930	1931	1930	1931
January	700	423	232	367	..	4	156	0	155	13	..	..
February	428	491	251	408	..	29	166	0	11	0	..	..
March	935	920	616	802	81	44	66	0	125	0	..	..
April	1,017	780	623	669	304	25	41	0	26	5	..	..
May	505	437	267	341	217	80	3	0	7	10	..	..
June	704	1,268	201	889	475	364	5	3	17	5	..	..
July	776	939	382	772	368	124	6	15	20	28	..	..
August	531	1,022	489	907	† 9	94	5	4	28	17	..	..
September	441	1,086	353	840	33	92	19	14	26	12	..	20
October	602	..	434	..	7	..	7	..	50	..	0	..
November	761	..	383	..	25	..	18	..	115	..	2	..
December	702	..	420	..	29	..	8	..	23	..	..	..
TOTAL ..	8,192	7,366	4,651	5,995	1,548	856	500	36	603	90	2	20

TABLE II—concl'd.

Months.	<i>A. hyrcanus.</i>		<i>A. barbi-rostis.</i>		<i>A. minimus</i> and <i>A. listoni.</i>		<i>A. aconitus.</i>		<i>A. vagus.</i>		<i>A. tessellatus.</i>	
	Years.		Years.		Years.		Years.		Years.		Years.	
	1930	1931	1930	1931	1930	1931	1930	1931	1930	1931	1930	1931
January	170	12	66	1	0	13	11	13	0	0	0	0
February	0	0	0	0	0	41	0	13	0	0	0	0
March	4	13	3	0	37	16	3	45	0	0	0	0
April	14	38	7	5	2	1	0	37	0	0	0	0
May	1	3	10	0	0	0	0	3	0	0	0	0
June	0	2	6	0	0	0	0	5	0	0	0	0
July	0	0	0	0	0	0	0	0	0	0	0	0
August	0	0	0	0	0	0	0	0	0	0	0	0
September	10	84	0	6	0	12	0	0	0	0	0	6
October	93	..	0	..	0	..	6	..	5	..	0	..
November	159	..	50	..	5	..	2	..	2	..	0	..
December	161	..	39	..	22	..	0	..	0	..	0	..
TOTAL ..	612	152	181	12	66	83	22	116	7	..	..	6



*Showing the number of mosquitoes caught, dissected and found infected with malarial parasites.*

	<i>A. fuliginosus.</i>						<i>A. pallidus.</i>					
Months.	Year 1930.			Year 1931.			Year 1930.			Year 1931.		
	Caught.	Dissected.	Found infected.	Caught.	Dissected.	Found infected.	Caught.	Dissected.	Found infected.	Caught.	Dissected.	Found infected.
January ..	7	::	::	::	::	::	::	:	:	::	::	::
February ..	1	::	::	::	::	::	::	:	:	::	::	::
March ..	::	::	::	::	::	::	::	:	:	::	::	::
April ..	::	::	::	1	::	::	::	:	:	::	::	::
May ..	::	::	::	1	::	::	::	:	:	::	::	::
June ..	::	::	::	::	::	::	::	:	:	::	::	::
July ..	::	::	::	::	::	::	::	:	:	::	::	::
August ..	::	::	::	::	::	::	::	:	:	::	::	::
September ..	::	::	::	::	::	::	::	:	:	::	::	::
October ..	::	::	::	::	::	::	::	:	:	::	::	::
November ..	::	::	::	::	::	::	::	:	:	::	::	::
December ..	::	::	::	::	::	::	::	:	:	::	::	::

[illegible]







## THE DEVELOPMENT OF MALARIAL IMMUNITY IN JALPAIGURI DUARS.

BY

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[April 28, 1932.]

IN an article dealing with certain peculiarities observed in the malarial temperature charts of patients living in the Chittagong Hill Tracts (Sarkar, 1930), the writer made the following remarks :—

‘After completing my spleen curve of the hill children of Chittagong Hill Tracts, I consulted Dr. Khambata, Assistant Director of Public Health Department of Bengal, as to whether a curve, similar to one obtained by me, can be constructed out of the materials of splenic census of children taken by his department for any place in Bengal. Dr. Khambata very kindly took time to ascertain this by actual trial with the statistical data available in his department and in the end gave his answer in the negative. Now what may be the reason that Dr. Khambata could not get a curve of splenic census like that of the Chittagong Hill Tracts even in the hyper-endemic malarial area in the plains of Bengal? The researches of Christophers have demonstrated the reason which is as follows. In the hyper-endemic area of Singbhum as well as that of Chittagong Hill Tracts there is happening acute infestation in consequence of which immune infestation is uniformly occurring amongst the hill children. But the hyper-endemic areas, which were under Dr. Khambata’s observation, were probably post-epidemic hyper-endemic areas, at least these were areas where the phenomena of acute infestation as well as immune infestation were not present in a degree to produce the phenomenon of immunity almost universally as was observed amongst the children of the hill tribes’.

These remarks were based on a conversation with Dr. Khambata, which took place about 10 years ago. Recently Dr. Khambata, now Director of Public Health, and Dr. S. N. Sur, Assistant Director of Public Health, Malaria Department, Government of Bengal, drew the attention of the writer to a

report on a malaria survey of the Jalpaiguri Duars (Sur and Iyengar, 1926), which contains much material of scientific interest. This report shows that the phenomenon of the development of immunity noted by me (Sarkar, 1921), and described by Christophers (1924), may also be observed in the Jalpaiguri Duars. Certain extracts from the portion of the report written by Sur are given below, with the permission of Dr. Khambata :—

‘The table shows the comparative spleen index which represents the percentage of enlarged spleens found in children who remained in the garden through several seasons, that is, amongst a population more or less stationary in the garden.

TABLE I.

Year.	With spleen.	Without spleen.	Total number examined.	Spleen index.
1919	111	40	151	73·5
1924	70	81	151	46·3
1920	159	23	182	87·3
1924	87	95	182	47·8
1921	149	66	215	69·3
1924	105	110	215	48·8
1922	144	87	231	62·5
1924	117	114	231	50·6
1923	153	114	267	57·3
1924	139	128	267	52·05

The above table shows that there are 151 children resident in the garden since 1919. Amongst them the spleen index was 46·3 per cent in 1924 against 73·5 in 1919—a great reduction. Again amongst 182 children common in 1920 and 1924, the spleen index was 47·8 in 1924 against 87·3 per cent in 1920; that of 215 children common in 1921 and 1924 was 48·8 per cent in 1924 against 69·3 in 1921. Amongst 231 children staying since 1922, the spleen index was 50·6 in 1924 against 62·5 per cent in 1922 and amongst 267 children the spleen index was 52·05 in 1924 against 57·3 in 1923. We find here that the longer the stay in this garden, the greater the fall in the spleen index. For those who stayed since 1919 the spleen index is 46·3. Hence, instead of the trend to rise, which is found in 1920, a trend to fall is noticed after 1921, that is after the extension of the area under operation’.

Here Sur has adopted a very interesting method for the study of the development of immunity, which had not been employed by any other malariologist, so far as the writer is aware. His results show that there was a rise

in the spleen rate for one year only, followed by a steady fall. This rise in the spleen rate for one year only is perhaps an indication of what has been termed by Christophers 'acute infestation'. The effects of this are checked by the gradual development of immunity.

The splenic index of children under 10 years of age at different age periods, has been tabulated in the report and is reproduced below, together with the remarks following it :—

TABLE II.

Age.	Total children examined.	Enlarged spleen	Percentages
1 year ..	337	272	80.7
2 years ..	307	269	87.6
3 „ ..	325	280	86.1
4 „ ..	281	239	85.0
5 „ ..	209	174	83.2
6 „ ..	307	241	78.5
7 „ ..	213	166	77.9
8 „ ..	245	187	76.3
9 „ ..	146	99	67.8
10 „ ..	584	370	63.1
TOTAL ..	2,954	2,297	77.7

'At one year or below, the spleen index is 80.7 per cent, at two years the index is the highest (87.6) and then it gets lower and lower as the age advances. Infants under one year have a lower spleen index than amongst those of two years not because they have more immunity, but that possibly they had not the chance of contracting the infection; children of two years are more exposed to infection and therefore the index at that age showed the actual rate amongst a non-immune population. As the age advanced, the immunity also increased and the children of 10 years age showed the lowest spleen index (63.1 per cent).'

The table reproduced above may be compared with one published by the writer (Sarkar, 1921), an abridged form of which is given in Table III.

Now if this table dealing with the Chittagong Hill Tracts be compared with that referring to the Jalpaiguri Duars (Table II) certain points of analogy will be at once manifest. In both the tables, there is rise of the splenic rate after the first year.

TABLE III.

Age in years.	Number of children examined.	Number of children with no splenic enlargement.	Number of children showing enlarged spleen.	Percentage of enlargement of spleen.
1 year ..	192	45	147	76.6
2 years ..	187	30	157	83.95
3 " ..	224	33	191	85.3
4 " ..	221	46	175	79.2
5 " ..	201	49	152	75.6
6 " ..	206	69	137	66.5
7 " ..	218	80	138	63.3
8 " ..	181	71	110	60.8
9 " ..	140	68	72	51.4
10 " ..	160	90	70	43.75
11 " ..	108	71	37	34.25
12 " ..	140	104	36	25.7
TOTAL ..	2,178	756	1,422	75.7

In the figures for the Chittagong Hill Tracts there is a gradual rise for the 2nd and the 3rd year. In the figures for the Jalpaiguri Duars, there is also a rise in the 2nd year, but in the 3rd year there is a slight fall.

In both places, there is steady diminution of the spleen rate for the subsequent years.

The figures of the spleen rate up to the 4th year in the case of Chittagong Hill Tracts and up to the 5th year in the case of the Jalpaiguri Duars are greater than the figure for that of the 1st year.

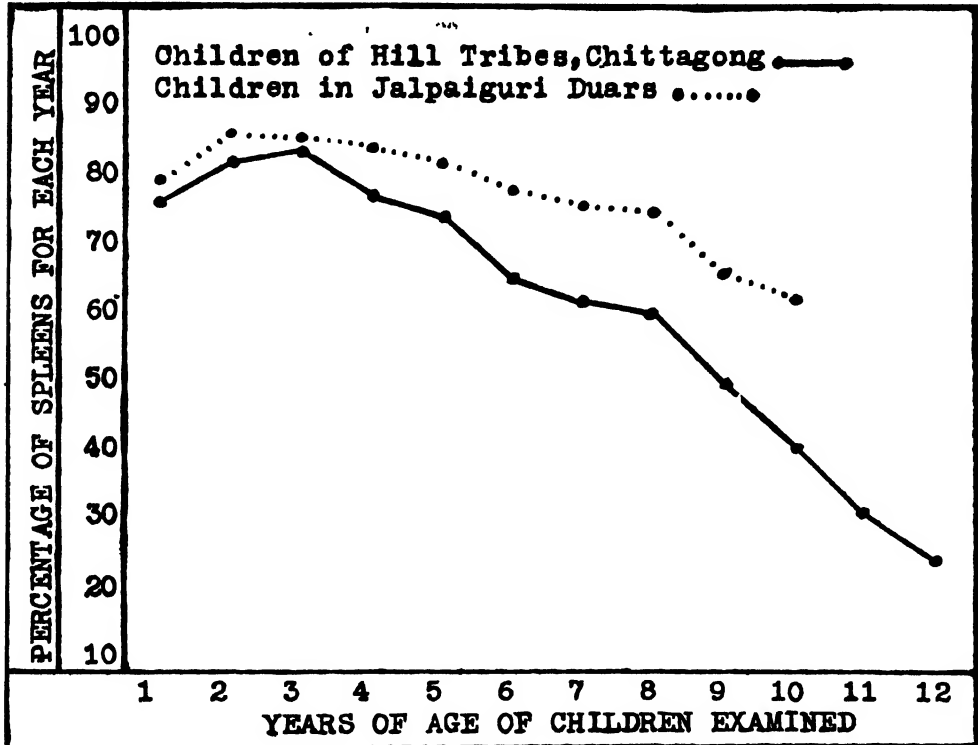
To demonstrate these analogies the spleen figures for the two districts have been depicted in the form of curves in the annexed chart.

From this it will be seen that the form of the two curves is of the same type, but the curve representing the splenic index of children of Chittagong Hill Tracts is much sharper than that of the Jalpaiguri Duars.

It may be that different races and different classes of people show some difference in the rate of acquirement of immunity. This would be a very interesting subject for investigation. For example, Sur noted that there were two classes of populations amongst labourers of the tea-gardens of Jalpaiguri Duars, viz., Paharias and Madesias, and that these showed certain differences regarding their susceptibility to malaria. For example Sur observes:—

‘It might not be out of place to mention, that, as a rule in all the gardens, Paharias have a higher spleen index than the Madesias. Amongst the Paharias

born outside or inside the garden, the spleen index is nearly the same, 86.5 and 85.6 per cent respectively. But amongst the Madesias, born outside, the spleen index (68.1 per cent) is much lower than that of the Madesias born within (79.5 per cent). The Madesia children, who have been shown as born outside the gardens, have apparently acquired some amount of immunity against malaria before entering the tea area and therefore suffer less. Paharias born outside possess no such immunity as they hail from a less or non-malarious place and they suffer as those born inside the garden'.



Now this observation shows that the development of malarial immunity in adult parents to a certain extent helps the development of immunity to malaria in children. This explains why there is more marked development of immunity amongst the hill tribes of Chittagong Hill Tracts than amongst the labourers of the tea-gardens in Jalpaiguri Duars.

#### *Conclusions.*

- (1) The phenomenon of immunity to malaria can be observed amongst the labourers of tea-gardens of Jalpaiguri Duars.
- (2) The curve of immunity here resembles that observed in the Chittagong Hill Tracts.

(3) Amongst the two different classes of labourers working in the tea gardens of Jalpaiguri Duars, viz., Madesias and Paharias, a distinct difference in susceptibility to malaria can be observed.

(4) This difference in susceptibility to a certain extent may be due to the fact that Madesias who come from malarious part of the district have already acquired some amount of immunity to malaria, which is not possessed by the Paharias.

(5) It has been found that amongst the Paharias born outside the garden, the spleen rate is 85.6 per cent but amongst the Madesia children born within the tea-garden, the spleen rate is 79.5 per cent. This lower spleen rate amongst the Madesia children born within the garden may be due, to a certain extent, to the influence of heredity of Madesia parents, as Madesias are found to have developed more immunity to malaria than Paharias.

(6) If the curve of malarial immunity of the Chittagong Hill Tracts be compared with that of the Jalpaiguri Duars, it will be found that the development of immunity is comparatively more rapid in the former area. This may be due to the fact that the parents of the hill-tribe children at Chittagong Hill Tracts have more marked development of immunity in them than that of the labourers of Jalpaiguri Duars.

I wish to express my thanks to Dr. Khambata, the Director of Public Health, as well as to Dr. S. Sur, Assistant Director of Public Health, for kindly giving me permission to utilize the materials in their department, arrived at by them, to write this paper.

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## AN INVESTIGATION ON PETROLEUM OILS FOR MALARIA CONTROL PURPOSES.\*

BY

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### Part I.

THOUGH different products derived from crude petroleum oil have been used for several years as larvicides in anti-malarial work, yet practical experience in the field has shown that there are great variations in the larvicidal properties of various petroleum oils. For this reason we considered that a further research on the action—chemical, physical and toxic—of the various petroleum products available in India was necessary in order that a highly efficient oil would be available for malaria control purposes. In addition to its use as a larvicide, petroleum oil has also for many years been used as an insecticide, but we shall refer briefly here only to its insecticidal property.

There are three main classes of petroleum larvicides and insecticides :—

- (1) Those which are spread on to the surface of water in order to destroy mosquito larvæ; incidentally also to burn vegetation, grass and weeds amongst which larvæ shelter.
- (2) Those which are sprayed into fine mists to destroy adult insects such as mosquitoes and flies.

\* A Research carried out in the Research Laboratory of the Burmah Oil Company, Ltd., at Syriam, Rangoon, and in the Southern Shan States during January and February 1932.

- (3) Those which, when emulsified with water by means of soaps, are used as sprays on fruit and other trees in order to destroy insect pests.

In addition to those obtained from petroleum sources, oils from lignites, shales, coal tar and vegetable sources may be used as larvicides, while certain directly poisonous substances in powder form, such as paris green, are suited for special classes of work.

Before dealing specifically with the larvicidal action of oil, it is necessary to consider the constituents of crude oils, as a clear idea of the problem cannot be obtained without a knowledge of the chemical composition of the various types of oil products.

While it is true that some limited measure of success can be obtained by an unscientific use of oils in the form of fuel oils, kerosenes and crude oils, it is obvious that a vastly greater degree of efficiency can be ensured by selecting those constituents which are most toxic to mosquito larvæ, and at the same time harmless to higher forms of life, those which spread well on water, and those which possess sufficient persistence to last economically, while yet possessing sufficient lightness and mobility to carry out their functions efficiently.

#### *The origin of petroleum.*

Petroleum occurs in oil-bearing porous strata ('sands') protected by impervious clays, and it is therefore associated with sedimentary deposits. Oils of somewhat different kinds are obtained from lignites, brown coal and shales by destructive distillation, while coal also produces oil under suitable treatment.

All these tars and oils contain many constituents in common though in different proportions, while some have constituents which are lacking in the others.

#### *The nature of petroleum.*

Crude oil is composed of a vast number of compounds containing the elements carbon and hydrogen, and is therefore an intimate mixture mainly of 'hydrocarbons'.

These are contaminated to some extent with impurities containing sulphur, nitrogen, oxygen and other elements; shale and coal oils also contain hydrocarbons, again associated with such impurities in greater degrees.

The carbon and hydrogen elements combine in a great variety of ways to form molecules, containing varying numbers of carbon and hydrogen atoms, any of which can be expressed chemically by a formula such as  $C_x H_y$ .

As  $x$  and  $y$  increase, series of compounds are possible, the individual members of which resemble each other in some measure, but all boil or melt at their own specific temperatures.

There are many classes of hydrocarbons, for not only does the number of carbon atoms in a molecule vary, but, with the same number, the ways in which they unite with each other and with hydrogen may give rise to various structures in the form of long chains, branched chains, rings or rings with side chains, each different structure giving rise to a different set of properties.

In any case crude petroleum oils differ very widely in composition, some being termed 'asphaltic', some containing one class of hydrocarbon predominantly, others other classes of these elements, so that in some cases certain crude oils are more suited for the preparation of anti-malarial oils than others, unless the latter are specially treated so that the correct constituents can be segregated for the purpose.

Crude oil contains four principal classes of hydrocarbons named respectively :—

- (1) Paraffins.
- (2) Unsaturateds.
- (3) Naphthenes.
- (4) Aromatics.

#### *Paraffins.*

Paraffins are 'saturated' and are comparatively non-reactive, hence their name (*parum affinis*).

The lower members in this class exist as the light bodies in natural gas and petrols, medium members in kerosenes and light fuel oils, while the higher members of high melting and boiling points are the solid paraffin waxes of commerce.

#### *Unsaturateds.*

Unsaturateds are very reactive and are easily oxidized or joined together (polymerized to resinous substances), so that their physical and biological actions may be marked. In chemical structure they are unstable and are therefore reactive, as there are free links unsatisfied. They will take up other hydrogen atoms, or will oxidize by taking on oxygen atoms, or the links will join up with the free links of another molecule.

Unsaturateds are usually liquid, but exist in whole series of products, from the light low-boiling volatile hydrocarbons to the heavy non-volatile viscous fractions. They are produced especially when heavy hydrocarbons are forcibly broken up by great heat ('cracked') into lighter substances.

Such oils have toxic properties, and spread well on water with high wetting power, a property connected with the tendency of the broken links to adhere to other substances with which they come in contact.

#### *Naphthenes.*

Naphthenes are mostly liquid, and chemically are more or less like Paraffins.

#### *Aromatics.*

Aromatics represent a highly important class of hydrocarbons which exist in petroleum, shale and coal oils alike. They are of high density (nearly that of water), and possess peculiar properties. In petrols, they diminish 'pinking' in motor engines, in kerosenes they tend, when in excessive quantity, to produce

smokiness on burning, while they are moderately reactive in many ways and possess excellent solvent qualities. They also have good toxic powers and good penetration effects on plants through 'wetting' except that their very high-boiling, heavier members are very viscous or sluggish at low temperatures and then are not useful for the same purposes for which the light or medium members give good effects.

*Main fractions from crude petroleum oil.*

When crude oil is distilled, the main fractions which boil off successively are themselves mixtures of all the above chemical classes, but are divided broadly into :—

(1) *Spirits* (Petrol or Gasoline).—A volatile range boiling from ordinary temperatures up to 150°C., 200°C. or higher according to grade.

They are mobile and colourless, and their use as internal combustion engine fuels depends on their ability to gasify with air to form an explosive mixture. This vapour is also toxic to all life.

(2) *Kerosenes or burning oils*.—These are heavier but still mobile liquids, almost colourless to yellow according to grade, and roughly represent those portions boiling from about 150°C. to 300°C. in the lighter grades or higher in heavier grades.

For safe transport they must possess a 'flash point' \* at least above a legal limit a little below 80°F.

(3) *Distillate 'Solar' and medium to heavy oils* are associated in the case of many crude oils with solid paraffin wax. These moderately heavy oils, according to their properties, may be used as Diesel and Furnace fuels and as lubricating oils, but in many cases must first be freed from wax by pressing at certain temperatures or by other means.

Unless wax is removed, some solidification may occur on cooling, and this is undesirable in most cases, including those of anti-malarial oils.

According to the use intended, these oils may be refined by further distillation, filtration, and treatment with chemicals or solvents.

(4) *Paraffin waxes*, separated from the last cut as just noted and specially refined.

(5) *Residues, 'back-ends', asphalts*.—These are the heavy dark products, which are left when distillates have been removed. Some Diesel and Furnace fuels represent 'long' residues of crude oil from which only petrol and some kerosene have been taken off.

Heavy residues can be subjected to intense heat treatment, when they break up or 'crack' to lighter oils containing high proportions of unsaturateds, in which condition they are very reactive.

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\* The flash point is the temperature to which an oil must be warmed at a certain rate to produce an inflammable mixture of vapour with air, which can 'flash'. This should be borne in mind in anti-malarial work when considering the high toxicity of spirits.

The methods by which the main cuts are refined depend largely on the nature of the crude oil, and whether it does or does not contain wax, gummy or asphaltic bodies; the presence of some classes of hydrocarbons may be more desirable in some cuts than in others, so that processes of segregation may be called for by methods of filtration, acid or clay treatment, solvent extraction, heat treatment and other means, whereby those special substances desired as solvents, anti-malarial oils and oils for special uses can be specially extracted or produced.

### *Physical properties of oils.*

In addition to the increasing boiling points and densities of distillates resulting from a distillation, other properties vary in the same way, e.g., viscosity and surface tension, two properties which are important to the malariologist.

*Viscosity* (that is resistance to flow or sluggishness) is a property which limits the *rate* at which an oil will flow or spread out, enter and travel along a capillary tube (such as the respiratory tubes of a larva). Viscosity increases rapidly as fractions become heavier and less volatile.

*Surface tension* is as important as viscosity. A liquid acts as though there were a stretched skin under tension trying to limit its surface, and it will, when in minute quantities at rest, try to gather itself into drops with the smallest surface for a given volume, unless there is some other force (generally gravity) tending to make it spread out. This 'surface tension' (or 'interfacial tension' between two liquids) is a definite force which can be measured and it depends on internal cohesion between molecules.

When a drop of oil falls on to the surface of water there are four forces to be considered:—

- (1) The force tending to keep the oil-air interface small.
- (2) The force tending to keep the oil-water interface small.
- (3) The force tending to keep the water-air interface small.
- (4) The force of gravity which pulls the oil down.

Now the increase of area produced by any spreading of oil caused by gravity is limited by the surface forces which attempt to prevent the oil-air and oil-water surface forces from increasing, but is obviously helped by the diminution of water-air surface brought about, hence an oil will spread more and more easily as the sum of (1) and (2) is less and less than (3)\*, while a low viscosity of oil will help to make the spreading more rapid and a high density of oil will provide a greater gravity force.

While most oils have similar oil-air values, the oil-water value varies greatly.

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\* If certain water-soluble substances, or those having a very highly adherent effect with water, are present, (2) is too greatly lowered, and the first few drops may spread at once, fouling the whole of surface and lowering (3) so much that further oil may not spread properly. This tends to complicate the situation when non-petroleum substances are added to oil, unless great care is taken.

Aromatics have high densities, while both they and unsaturateds, particularly the latter, have lower interfacial tensions with water than those possessed by paraffins.

This is also true in a modified sense when considering the 'wetting' effect on solids such as vegetable tissues, insects or larval tubes, so that they are good spreaders and at the same time good 'wetters' and show high toxicity.

If the oils are too viscous, this effect is spoiled, hence it is necessary to keep to the right ranges of products. When the viscosity is too high, the forces tending to deform any existing condition of oil may be altogether upset.

Oil-water interfacial tension can be lowered greatly by the addition of soaps, alkalies, unsaturated vegetable oils or fatty acids, oxidized and resinous bodies.

But this effect must not be overdone, particularly with water-soluble substances, owing to a tendency with low enough interfacial tensions (1) for the first few drops to spread to too thin a film at once, partially dissolving and lowering the tension of the whole area so much that the remaining oil cannot spread out, and (2) to favour the formation of oil-in-water emulsions, thereby causing waste from oil films through the introduction, through dispersion, of minute globules throughout the aqueous layer; these, under such conditions, produce a toxic effect on fish and other natural enemies of larvæ. With a simple oil layer without emulsification, fish may be able to swim out into the open spaces away from the oil film near the banks of a stream or pool.

Soaps and alkalies therefore should, if present at all, be introduced into an anti-malarial oil only in very small proportions, particularly when it is remembered that the detergent action of a soap is based on this very tendency to remove fatty and oily substances.

#### *Tree sprays.*

For tree sprays, however, a soap-oil-water emulsion is just what is required.

In these, an oil comparatively non-toxic to leaves should be used in dilution with water as an emulsion, such that the oily globules will still destroy pests such as aphids without injuring the foliage.

Thus it is seen that some opposite properties are required for anti-malarial oils as contrasted with tree sprays, for efficient anti-malarial oils should assist in destroying vegetation which shelters larvæ.

#### *Necessary properties of anti-malarial oils.*

The main properties to be considered are therefore those of density, volatility, viscosity, surface effects and toxicity, with certain other economic factors.

We may classify the desired properties of an ideal anti-malarial oil, which should :—

- (1) yield a film which spreads well and covers surfaces without gaps at a reasonable yield (say 4 to 5 ounces per 100 square feet);

- (2) possess sufficient persistency to remain for several days as a homogeneous and effective film without separation of solids;
- (3) be sufficiently low in viscosity to be sprayed effectively and to enter larval breathing tubes with ease;
- (4) wet and destroy vegetation under which larvæ shelter;
- (5) be highly toxic in the chemical sense to mosquito larvæ, but not to fish, nor must it unduly inconvenience birds and animals;
- (6) be capable of covering algæ in a stream with oily 'felting', and thereby reduce the food supply of mosquito larvæ; and
- (7) not be desirable as a burning oil on account of the possibility of theft by coolies.

*Experimental work and results.*

Summarizing the work which has been carried out in the laboratory and in the field, it has been found that :—

- (1) For a given class of oil, the more volatile and less viscous the oil, the more rapid is the killing effect on mosquito larvæ. Pupæ are somewhat difficult to kill, though petroleum spirits do this quickly, even in vapour form over the surface of water.
- (2) The above must be correlated with desired persistence. Petrol is almost immediate in its action on all larvæ, while light kerosenes are good, but when the oil is sufficiently volatile to kill almost at once, the film is evanescent; apart therefore from special cases of emergency against which the fire risks and cost of the volatile product are not of importance in relation to the immediate effect desired, it is necessary to utilize a less volatile product.
- (3) Apart from the consideration of the chemical toxicity of specially prepared oils, the compromise between volatility and persistence comes into medium ranges after light products have been removed.
- (4) Such oils of correct persistence must also have good mobility (low viscosity) for good biological effect, and, of course, they should be good spreaders.

Expressed in certain scientific units (centipoises at 25°C.) the viscosity should usually lie between 3 and 15 units, preferably nearer the lower limit, but should not be high.

(Note that water and petrol give values below unity, light kerosenes under 2, heavy kerosenes 4 to 8, solar oils 6 to 12, heavy fuels and lubricants 15 upwards.)

It will be seen later that killing effect is definitely related, not only to asphyxiation through the oil entering the breathing tubes, but to the entry of toxic substances *both* into the breathing tubes and mouths of mosquito larvæ; hence the ease of entry, which depends both on viscosity for its rate and surface tension for further effect, must be good.

Anopheline larvæ, which lie along the surface of water and possess two respiratory spiracles opening on their dorsal surfaces, can be more easily killed than culicine or *stegomyia* larvæ, which hang down with a long breathing tube in the form of a tail which penetrates the surface.

Before the mosquito larvæ can be destroyed in their breeding places by oil, it is essential that either toxic constituents or oil must enter their respiratory siphons, for larvæ can survive for several hours on dissolved oxygen in water. This we demonstrated by introducing larvæ (anopheline, culicine and *stegomyia*) into bottles containing ordinary tap water, which were completely filled and hermetically sealed, so that no air could enter and no space was available for the respiratory spiracles to function. These larvæ survived for several hours, but when the experiments were repeated with de-aërated water, we found that all larvæ succumbed within twenty minutes. Chemical toxicity is therefore of the utmost importance for an anti-malarial oil to function effectively on breeding places such as running streams.

Our work confirms that of Ginsburg (1929), in which direct evidence of the entry of dyed oil into the respiratory tubes has been obtained microscopically, and draws attention to the importance of viscosity and surface tension as factors upon which the preliminary nature of the oil affects the exertion of the full toxic effect.

- (5) Preliminary physical properties being adjusted to correct ranges in accordance with what has been said above, it is found that aromatic and unsaturated oils, extracted or produced from certain ranges or 'ends' of oil and blended in correct proportions, approximate closely to the ideals discussed above.

They are abnormally toxic to weeds, insects and larvæ; they possess good wetting and spreading power, owing to properties connected with their chemical activity.

They kill the majority of anopheline larvæ in two to five minutes, against half to one hour for ordinary oils; even *stegomyia* larvæ are killed within twenty minutes, as against several hours with ordinary oils; at the same time, unless these oils have been shaken vigorously with water, fish are not killed under them.

- (6) The effect of the addition of such substances as vegetable oils or acids, turpentine, creosote, oxidized aromatic bodies such as phenol and cresol, alkalies, and tars has been studied, but nearly all of them increase the cost, and in most cases the effect produced is not sufficiently marked to be worth while.

Some workers have commented on improved effects brought about by vegetable oils such as castor oil, but the increased spreading effect is generally very little, and these oils are not toxic. Turpentine, being an unsaturated light oil, is highly effective, but it evaporates quickly and its cost is high. Fatty acids may cause the first drop



or two to spread quickly, dissolve partially and spoil the surface for the bulk of the oil which will then remain rolled up in blotches. This is the danger with many substances which are soluble *both* in oil and water.

Phenol (carbolic acid) and cresol are highly toxic, but they are water-soluble and their presence is dangerous to fish.

Crude creosote oil gives some advantage in the burning of weeds, and may be used in proportions of 2 to 5 per cent to anti-malarial oils to add to their effect; such creosote should not be appreciably soluble in water, and when incorporated in oil will not then hurt fish unless swallowed.

### *Sprayers.*

The immediate film produced on a water surface depends to a great extent on the efficiency of spraying and the sprayer used. Vermorel Eclair and 'Ross' Four-Oaks Sprayers are efficient when used correctly, but the flexible connections in these sprayers should be of metal, not of rubber, as rubber rapidly perishes in oils.

It is important that sprayers should always be kept in good condition, with no dirt or obstructions in the nozzle. The spray should emerge in a good fan-shaped manner directed upwards, for when the oil falls on to the water, a much better film is produced than when the nozzle is aimed directly at the water, producing blotchy effects. This may be important, particularly when an oil has some tendency to remain rolled up into blotchy patches.

The amount to be used should be about four or five ounces to 100 sq. ft., or sometimes more than this; one gallon should cover about 250 linear yards with a spray one foot wide, allowing for the fact that the oil will spread much more after it has reached the surface. When a running stream is sprayed, it should be given somewhat more than the above allowance, and spraying should be carried out by walking up *against* the stream. At all times a man using the sprayer should walk briskly in order to avoid waste of oil; *this is all largely a matter of experience.*

Of the literature on the subject we refer especially to the following papers, which bear directly on our remarks :—

Gray and de Ong (1926) and de Ong (1928a, 1928b, 1930) have discussed the use of oils for tree spraying, and conclude that unsaturateds are more toxic to foliage than saturated substances. For this class of oil they therefore recommend refined oils free from unsaturateds. (*Note*—The reverse function is required in anti-malarial oils, as noted above.)

Shutt (1928) adds minute quantities of caustic soda solution to a spray fluid, in order to improve the continuous nature of an oil film on water.

Inman (1929) concludes that sulphonated oxidation products of petroleum act as activators of other toxins in petroleum sprays, owing to the increased

wetting power introduced. (Note—Equivalent to the action of soapy substances.)

Ginsburg (1928) concludes that the most toxic fractions of petroleum, combined with lasting power, are those boiling over the range 350–740°F. (approximately 175–400°C.), and that the volatility of the lighter fractions present must not be masked to too great an extent by an undue proportion of heavier, though some of the latter are required to 'fix' the lighter constituents.

The same author (Ginsburg, 1929) examines the toxicity of oil fractions boiling between 200°F. and 700°F., and concludes, from an observation of the penetration of oils into the breathing tubes of larvæ with the aid of a microscope and oil-soluble dyes, that toxicity is directly proportional to the volatility and inversely to the boiling point. Oils of low boiling point (200–500°F.) kill in 30 minutes or less by direct toxic effect, whereas higher boiling point oils cause death by suffocation at a rate proportional to the thickness of the film; respiratory siphons of many larvæ and the trumpets of pupæ can penetrate through thin oil films.

When the respiratory siphons are filled with non-toxic oils, the larvæ do not develop into pupæ.

#### *Insecticide sprays.*

So far little mention of this class of oil has been made. Insecticide sprays for the control of adult mosquitoes, flies and insects are based on light oils, sufficiently non-volatile to be safe from undue fire risk, but sufficiently volatile to evaporate from fabrics in a short time, without leaving a stain or residue.

Toxic substances, together with odorants, are added to the main base subject to the above requirements, and to the proviso that the effect must not be harmful to humans and animals. Thus the properties of these oils are quite different from those either of anti-malarial or of tree-spraying oils.

## **Part II.**

### **EXPERIMENTAL VALUES.**

The action of air, silt, temperature and oils on mosquito larvæ, including some observations on adults.

EFFECTIVE anti-malarial measures including oiling depend on a knowledge and choice of the best conditions at which to carry on the work. A few conditions have been investigated as follows :—

#### *Effect of absence of oxygen dissolved in water.*

De-aerated water, entirely freed from oxygen, will not support the life of larvæ for more than a few minutes.

Various small quantities of air-free water (checked for air-freedom by Winkler's method of titration, using manganous salts) were prepared :—

- (1) by continued boiling, followed by cooling out of contact with air,

- (2) by the action of confervoid organisms, followed by percolation through iron scrap (turnings),  
 (3) by being drawn from an enclosed plant system which normally operates on oxygen-free water.

The water samples were introduced into air-tight glass-stoppered bottles, with displacement of all air by means of continued flow of the samples; larvæ and/or pupæ were then inserted carefully and the whole stoppered tightly, with inclusion of no air bubbles.

As blanks, ordinary tap water, also original samples of (1) and (2) not de-ærated, also the de-ærated samples (1), (2) and (3) through which air was blown to saturation, were used under identical conditions.

Results below are given as the intervals after which larvæ or pupæ were all found to die.

		<i>In air-free water samples.</i>	<i>In tap water and original non-de-ærated samples.</i>	<i>In de-ærated samples (1), (2) and (3).</i>
Pupæ	..	All less than 20 minutes.	50 mins.	20 mins.
<i>Anopheles</i>	..		45 hours	41 hours
<i>Culex</i>	..		41 hours	40 hours
<i>Stegomyia</i>	..		18 hours	17 hours

These results are very instructive and show that the drowning of larvæ may be a slow process when air is dissolved in water. If the water in a pond is deficient in dissolved oxygen, owing to the presence of certain fungoid organisms of the types of *Beggiatoa alba*, *Sphaerotilus*, *Leptomit*, *Crenothrix* and other growths which absorb oxygen from water, even a non-toxic oil will cut off the surface air supply needed by larvæ in order to exist for any appreciable time.

Many of these confervoid growths exist in surface drainage water in the tropics, and can be utilized under control for the de-æration of water as an anti-corrosive measure.

#### *Effects of silt.*

That silt has a larvicidal action can be shown in the laboratory by passing a gentle stream of air bubbles through silty water to keep the water turbid, as normally all silt, visible to the naked eye, is deposited from suspension in stagnant water within 13 minutes.

In a series of experiments of this nature, where several hundred *A. minimus*, *A. philippinensis*, *A. fuliginosus*, *A. hyrcanus* and *A. barbirostris* larvæ were introduced into jars containing water with a high percentage of silt (Rangoon river water), 50 per cent of the larvæ died within 24 hours, 90 per cent within 48 hours, 97 per cent after 100 hours, with all dead within 110 hours. The

dead larvæ were subsequently recovered from the silt-laden water by passing it through a fine wire-mesh sieve.

*Effects of temperature.*

(1) *Cold*.—It has been demonstrated that certain species of mosquito larvæ, in countries where the water becomes converted into ice, can lie under water for long periods in a state of suspended animation, and recover when the temperature of the water is again raised.

To test the effects of low temperatures, several types of mosquito larvæ which were collected around Rangoon were introduced with their own pond water with weeds and plenty of food into bottles, and were then placed in cold rooms kept at 50°F. and 30°F.

With larvæ existing naturally under the equable conditions of Lower Burma (average annual mean temperature 80°F., extremes 60°F., and 108°F., with water which does not reach these atmospheric extremes) it was found that at 50°F., anopheline and culicine larvæ died within four days, but 50 per cent of *stegomyia* larvæ were still alive after that period. Of pupæ of various kinds, 50 per cent died, 25 per cent hatched and 25 per cent were alive in their original form after four days. After seven days a few pupæ and *stegomyia* larvæ were still alive.

At 30°F. several *stegomyia* larvæ were alive after 8 hours, but all died within 24 hours.

When ice blocks were placed in water, all the larvæ and pupæ fell to the bottom as the temperature fell below 60°F., but though many larvæ died of shock, several recovered when the ice had melted and the water again reached 65–70°F.

(2) *Heat*.—Neither larvæ nor adult mosquitoes can live at high temperatures.

This has been investigated by such experiments as these :—

- (a) Water, in which various kinds of larvæ and pupæ were present, was warmed over a bath at a rate of 1°F. per five minutes, as measured by standard thermometers placed in the vessels.
- (b) A large (6 cubic feet) glass-sided cupboard was fitted for heating purposes with a tube which passed through it from bottom to top. This tube was heated by means of a burner, with no possibility of any burned gases entering the chamber; a small fan was also placed in the cupboard to keep the temperature constant by air circulation.

Various beakers and basins fitted with thermometers were placed on stands in the chamber away from the neighbourhood of the warm tubes; these beakers contained larvæ and pupæ in water. One of the basins contained pupæ which were allowed to hatch out into adults for the adult tests.

The temperature inside the chamber was also registered by several thermometers hung in various places.

The following results were obtained :—

		First died at	All were dead at
Anopheline larvæ	..	107°F.	111°F.
Culex larvæ	..	109°F.	111°F.
Stegomyia larvæ	..	109°F.	109°F.
Pupæ (various)	..	105°F.	107°F.
Adult mosquitoes	..	120°F.	125°F.

When heating was sufficiently slowly carried out, nearly all died within two degrees of the lower limits given above.

#### *The effects of various oil products.*

The effects of the main classes of oily products have been discussed in a general sense in Part I.

A very large number of trials, both in the field and the laboratory, have been carried out.

The laboratory trials consisted mainly of series of toxicity determinations carried out in each case in large open porcelain basins with 100 larvæ (all about the same size) placed in water such that a circular surface, 8 8 inches in diameter, was exposed. The oils were spread on to these surfaces in quantities equivalent to 5½ fluid ounces per 100 square feet, or 0 7 ml. per basin.

This was taken as a convenient standard quantity comparable with that used from 4 oz. per 100 sq. ft. upwards to 1 oz. per 15 sq. ft. or 6·7 oz. per 100 sq. ft.

The investigations were taken in sequence from light to heavy ordinary straight distillates and residues of crude oil, then from light to heavy specially prepared aromatic and cracked oils, finally to blends of these with heavy fuel oils. In addition some experiments were carried out in which small glass dishes containing water and larvæ or pupæ were placed on a stand above a dish of petrol or other oil, the whole system being enclosed in a bell jar.

A few examples from the very great mass of data obtained are given, but a complete tabulation would perhaps be out of place.

As already stated, the lighter and more volatile the oil (with petrols giving the light limits), the more immediately toxic is the oil for similar chemical classes. Light oils enter readily into the larval respiratory spiracles and breathing tubes, but the lightest (and therefore most toxic) do not give sufficiently lasting effects. When the average boiling point (the temperature at which 50 per cent of the oil distils) is over 200°C, the toxic effect diminishes appreciably, yet it is necessary to prepare oils with sufficiently stable films from cuts of higher boiling point than this.

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## MALARIA SURVEY OF TWO TEA ESTATES IN UPPER ASSAM.\*

BY

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### ERRATA.

Page 227, line 5, for 'being' read 'begin'.

Page 237, line 10, for '10,154' read '40,154'.

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and various expenditures. The malaria research work of the Assam Medical Research Society worked here during June, July and October 1931, and January 1932. The dissections were done by Dr. B. K. Das of the Society, under the supervision of the Research Officer, and, during his absence, of the Medical Officer of the Estates in question. One collector was stationed on Estate 'B' and catches were sent by him daily to the Field Laboratory at Estate 'A'.

### A. Topography.

The following is a short description of the areas involved :—

#### ESTATE 'A'.

This garden is situated in the plains of Upper Assam, about a mile from a large tributary of the Brahmaputra River, and about four miles from the foot of the Naga Hill range. It is about 300 feet above sea level. The special features affecting the malarial problems are noted for various coolie lines separately.

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\*The expenses of this survey were borne by the Assam Medical Research Society.

*Main lines.*

These are situated on a triangular-shaped piece of ground, of which the base is formed by the road to the factory, and the sides by main drains which unite at its apex. From this union, one main drain extends into a strip of low-lying rice land, on to which it empties its water at a distance of half a mile from the lines. These lines are close to and parallel with the road, on the opposite side of which stands the hospital.

About 250 yards from the centre of these main lines are the following :— (a) to the south-east, grouped together, the Superintendent's and Assistant's bungalows and factory; (b) to the south, the Christian lines; (c) to the south-west, the beginning of Line 21; (d) to the west, undulating land traversed by the main drain towards the boundary and jungle; and (e) to the north-west, at somewhat greater distance, the beginning of Line 17. In all other directions there is tea cultivation, intersected by its large and section drains. The land immediately adjoining the lines to the west, and that dividing the main and Line 21, is rough, and up to 1928 was covered with jungle. This land is low-lying and full of depressions, and in the rainy season it is largely under water. During the last three years Naga labour has been employed, in the winter months, in cutting this jungle down. This policy of annual clearance is fraught with danger from the malaria point of view, as it provides optimum conditions for the breeding of the dangerous species of anophelines. If clearance were complete and depressions filled it would be an improvement. Beyond the boundary fence, to the west, there is a narrow strip of Government land covered with patchy jungle, which separates the garden from the low-lying land which is cultivated for rice. Immediately to the south of the Christian lines is a small area of rice land, and two ponds which gradually are being filled in with refuse. These latter lines are close to the factory.

*Line 21.*

This long line is well spaced out, with forty to fifty yards between each house. It is on the edge of the grant, the line being between the tea and the boundary fence. A narrow belt of bamboos separates the tea from the line at the southern end, and the rough ground, previously described, separates it on the northern. There is a large Assamese village immediately on the other side of the boundary fence. The K— stream lies close to the southern end of these lines and winds behind the village. The drain running along the boundary fence is fairly well shaded with ferns. There are many borrow-pits between the houses and the road by the boundary fence.

*Lines 15, 16 and 17.*

These lines are scattered along the edge of low rice land and are interspersed with occasional Assamese houses. These lines are small and are occupied entirely by Oraons.



*Line 20.*

This is situated about a quarter of a mile to the east of the factory, and is occupied by 'short-term' coolies. This site, from every point of view, must be considered the most unhealthy of any on this estate. Surrounding the houses is boggy waste land, partially covered with vegetation, and on one side there is a small strip of jungle. From this low-lying boggy land the aforementioned K— stream arises.

## ESTATE 'B'.

This garden is about seven miles from Estate 'A', on the banks of the same river, which bounds it on three sides. On the fourth side is the Forest Reserve. This garden is in the foot-hills of the Naga range, at a height of approximately 350 feet above sea level, and is partly on rock, ten to twenty feet above the bed of the river. Water drains away quickly, except at the time of the highest floods, and there is a gentle flow in the perennial streams throughout the cold weather. The land is intersected by deep ravines and narrow streams. The beds of these streams are of gravel, and their margins, for the most part, are covered with vegetation. In many places this vegetation was completely overhanging the streams, but unfortunately it has been cut. Many of the ravines are naturally covered with dense vertical vegetation (*tarapat*, etc.). The irrigation ditches in the tea drain into these ravines and, except in heavy rains, these keep dry. The lines were about two feet above water level in the highest flood of the year under survey. Rice is cultivated in a few places on the periphery of the estate, e.g., near the Saura lines. There is a channel of water flowing through this land, which encourages the breeding of dangerous species of anophelines.

**B. Seasonal difference in rainfall.**

The total annual rainfall is about 110 inches, the heaviest precipitation usually being between the months of May and September, with lighter rainfall in other months. The fall in December and January of the last five years has been low in relation to that of the other months. The hot weather extends from June to October. Throughout the year the air is practically saturated, relative humidity ranging from 85 to 95 per cent.

**II. MALARIA SURVEY.***(a) Larval catches.*

We surveyed these estates and the neighbouring villages, collecting specimens from 82 different breeding places on Estate 'A' and some 25 on Estate 'B'. Where time permitted these were identified as larvæ, otherwise they were hatched out and pinned and then identified as adults. All species caught in the adult form were also found as larvæ and, in addition, *A. stephensi* and *A. subpictus* were found as larvæ only. Of the important carrier species,

*A. culicifacies*, *A. maculatus*, *A. stephensi*, *A. philippinensis* and *A. minimus* have been found. The first three species mentioned occur in such small numbers on Estate 'A' as to appear of little importance. The same applies to Estate 'B' with the exception of *A. maculatus*, which was found in considerable numbers throughout the season; *A. maculatus* was found only in June in Estate 'A', whereas in Estate 'B', in the low hills, it was found during every month of the investigation. This is in accordance with findings elsewhere, *A. maculatus* being primarily a hill-stream breeder.

*A. culicifacies*, which was a species suspected of carrying malaria at Mariani by Macdonald and Chowdhury (1931), was found on Estate 'A' in drains, in rice fields, in ditches and in wells, but in very small numbers, and in the months of August and November only. Whether this be a carrier or not, in Assam has not yet been proved, but it is a dangerous vector in other parts of India. Assam appears to be the eastern limit of its appearance.

*A. philippinensis* occurs plentifully in many situations. Owing to the domesticity of habit of the adult, and the fact that two specimens have been found infected in Assam by us, it appears to be of some importance. This is a common carrier species in Bengal.

*A. minimus* was recovered, for the most part, from streams, drains and pools; in addition one was found in a rice field and two in wells. On Estate 'A' our biggest catches of *A. minimus* were from the marshy land adjoining Line 20, whereas it was caught in all streams in Estate 'B'.

Of the other species, *A. aconitus*, which generally is more plentiful in the cold weather in Assam, was found here only in November. *A. tessellatus*, *A. leucosphyrus*, and *A. subpictus* were found in extremely small numbers. *A. hyrcanus* var. *nigerrimus*, *A. kochi* and *A. vagus* were recovered from all situations, and very frequently from extremely dirty water, the two former being obtained from water red with iron oxide from a scrap-iron dump.

#### (b) Adult anophelines.

The following 15 species of anophelines were identified :—

<i>aconitus</i>	<i>jeyporiensis</i>	<i>pallidus</i>
<i>barbirostris</i>	<i>kochi</i>	<i>philippinensis</i>
<i>culicifacies</i>	<i>leucosphyrus</i>	<i>ramsayi</i>
<i>fuliginosus</i>	<i>maculatus</i>	<i>tessellatus</i>
<i>hyrcanus</i>	<i>minimus</i>	<i>vagus</i>

Catches were made regularly in coolie houses, babus' quarters and cattle sheds. There is often difficulty in collecting in lines, as actual numbers of anophelines are relatively few, and coolies do not like their houses entered, except when they are at home in the morning or evening, during which time the mosquito is often driven out of the house by the smoke of cooking.

Most of the *A. minimus* in Estate 'A' were caught when actually in the act of biting coolies. Here the commonest species in houses were *A. vagus*

and *A. philippinensis*. The latter represented one-quarter of the total catches in July, August and October, but only 1/50th in September. This latter paucity of numbers appears to have no relationship to rainfall, which was heavy throughout these months. In November the proportions were 1/14th and in December 1/300th, only one specimen having been caught in the latter month. In Estate 'B', *A. vagus* and *A. kochi* were the two species found in the greatest number in the houses. Many specimens of *A. maculatus* were caught in cattle sheds.

Our catches of *A. minimus* were very small all through the rainy season, and it was only in October that we began to find them in numbers. As will be seen under 'Infectivity', the total number obtained for dissection in the last three months of the year in Estate 'A' was only 100. This cannot have been due to poor work on the part of the collectors, as experienced men were stationed on both estates from June onwards, and altogether twelve men have been engaged in this work from time to time.

As constantly throughout this period these various people had been urged particularly to collect *A. minimus*, it is apparent that actual numbers of this species were very small until the end of October. In November, however, we obtained a very rapid increase in the catches on both estates. This means either a large multiplication of the species, or that less have been washed away and destroyed, or that they have changed their habit and remain longer in the houses through cold. As this rise at the end of the year agrees with the findings of Manson (1931), it seems to us very important that collections and dissections be continued throughout 1932 and this observation confirmed. If confirmed, then control might only be required on these estates during the latter months of the year and excellent results might be expected with relatively little expenditure.

Some specimens, sent to Kasauli for confirmation of species, were reported upon as possessing unusual markings, differing from both *A. philippinensis* and *A. pallidus*.

#### (c) Infectivity survey.

To ensure time for the development of oöcysts, mosquitoes were kept alive for a week or so feeding on currants, and suitably protected from ants. The total number of dissections from Estate 'A' was 2,253, while from Estate 'B' there were 1,146 and from the district round about an additional 427. The process of dissection and microscopical examination necessary to determine the vector is most tedious, one worker seldom doing more than 30 to 40 mosquitoes per day. *A. minimus* and *A. philippinensis* were the only two species found infected, and of these we consider the former to be the principal carrier of the disease.

In Estate 'A', only one specimen of *A. minimus* was found before November and this was uninfected. In November three specimens were found

infected out of 47; in December, one infected out of 16. The infectivity rate for *A. minimus* on this estate was therefore 6·2 per cent during these months. This rate is high, even allowing for the large mathematical probable error in calculation from such small numbers.

In Estate 'B', a total of 572 *A. minimus* were dissected, and similar results obtained, there being no *A. minimus* catches until October and then only one infected out of 29 caught. In November eleven were positive out of 269 dissected, and in December four out of 274. From this much larger number, the percentage of infections works out at 2·79. Whatever relation the numbers caught may bear to actual numbers penetrating houses, the results appear to show the increased prevalence of *A. minimus* in the latter months of the year, and the extreme importance of control measures during that period particularly.

An important piece of knowledge gained from this survey is the infectivity of *A. philippinensis*. This is a carrier in Bengal, but has not previously been reported infected in Assam. Only one specimen out of a total of 560 dissected was found positive in these estates, representing an infectivity rate of only 0·02 per cent, but this same year an infected specimen was caught by us on a patient in Goalpara District. Both were infections of the gut and not of the salivary gland, so it may be that in this species parasite development in the mosquito proceeds no further in this locality of Assam.

As it is possible that *A. philippinensis*, or other species carry malaria here in the earlier part of the year, it seems to us important that further dissections of all species be made. If *A. minimus* alone be found infected, and that only at the end of the year, then efforts can be concentrated at that time on the destruction of this species.

Of the other species showing domesticity of habit, no specimens of *A. vagus* have been found infected, though 661 have been dissected to date from Estate 'A', and a total of 1,007 from the district.

Flagellates were found in specimens of *A. vagus*, *A. barbirostris* and *A. philippinensis*, but these flagellates were parasites of cattle and not of man.

#### (d) Spleen survey.

*Estate 'A'.*—The spleen rate, i.e., the percentage of children between the ages of two and ten years found to have enlarged spleens, in the various lines was as follows :—

Main Line.	Line 20.	Line 21.	Lines 15, 16 and 17.
55	73	67	63

These were taken by ourselves. The combined rate of all lines taken together was 61 in June 1931, when 175 children were examined, and 58 in December 1931, when 258 were examined.

The figures show the need for active anti-malarial measures.

*Estate 'B'.*—The spleen rate obtained in this garden was 82, the number of children examined being 87. This high figure occurred with a parasite rate of 68.

Both estates are thus areas of hyperendemicity.

(e) *Blood survey.*

*Estate 'A'.*—Of slides of the peripheral blood from one hundred children examined, 59 were found infected with malarial parasites.

The proportion of benign tertian and malignant tertian infections was about equal, the percentage of malignant tertian being slightly lower than on Estate 'B'.

Nine benign tertian films showed schizonts and two showed gametocytes.

The number of gametocyte carriers was fewer than we expected, since examinations were made at the end of the year, i.e., at the close of the transmission period.

*Species incidence.*

<i>Species of parasite.</i>		<i>Number positive.</i>
<i>Plasmodium falciparum</i>	..	.. 31 (including 5 mixed infections).
<i>Plasmodium vivax</i>	..	.. 33 do.
<i>Plasmodium malariae</i>	..	.. nil

*Estate 'B'.*—The parasite rate on this estate was 68 positive in the 100 children examined. The proportion of malignant tertian infections was slightly higher than benign tertian, and on this estate ten quartan infections were found, although none were found on the lower situated estate. Eight of the benign tertian and ten of the quartan films showed schizonts. Five malignant tertian and one quartan infection showed gametocytes.

*Species incidence.*

<i>Species of parasite.</i>		<i>Number positive.</i>
<i>Plasmodium falciparum</i>	..	.. 33 (including 2 mixed infections).
<i>Plasmodium vivax</i>	..	.. 27 do.
<i>Plasmodium malariae</i>	..	.. 10

### III. STATISTICAL FINDINGS.\*

(a) *Death rate per mille.*

*Estate 'A'.*—It will be seen from Graph 1 that the death rate per mille has increased from 10·81 in 1926 to 28·53 in 1931. The increase in the number

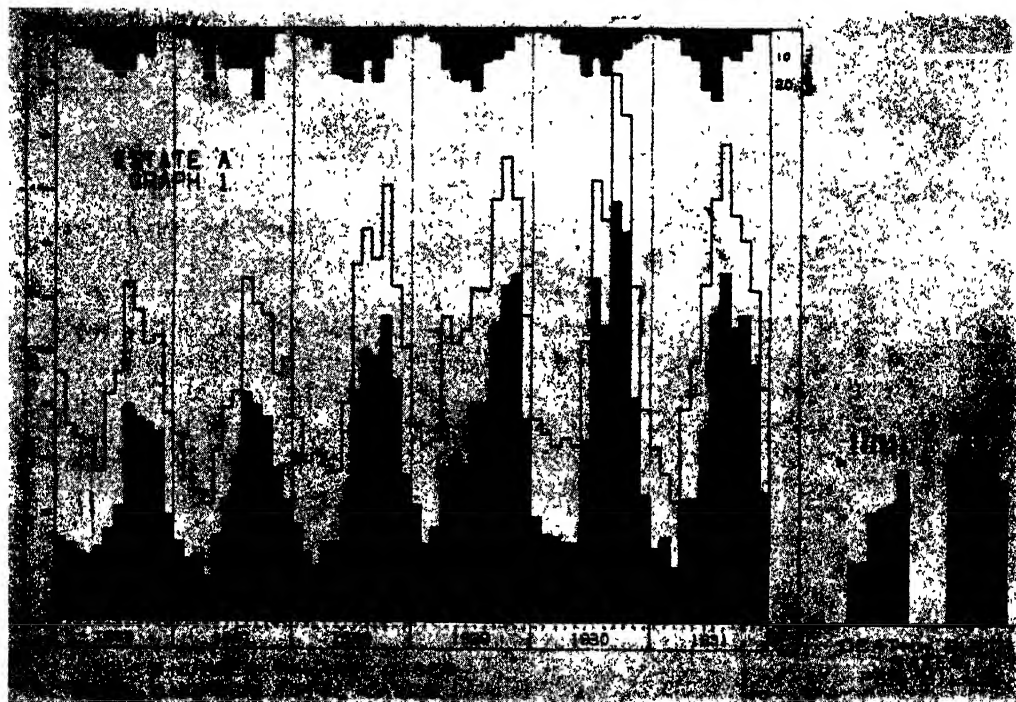
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\* On Estate 'A' about 20 per cent of the labourers have short-term contracts for periods ranging from one to two years. Most of these coolies come from areas where there is little malaria; hence, in this hyperendemic area, they are termed 'non-immunes'.

of deaths has been principally among the children over one year of age and among the adults; the infant deaths being comparatively the same throughout the period. Such an increase in the child and non-immune adult death rate is compatible with the increase in the malaria incidence as is definitely shown in the graphs for this estate. While a death rate of 28.53 per mille is not extremely alarming, approximating the average for the province, the increase from 10.81 to 28.53 in the short period of six years suggests that the increase may continue unless some measures be taken to stop it.

During the period there was a total of 167 deaths from all causes (other than still births), of which 39 or 23.37 per cent were directly due to malaria.

GRAPH 1.



*Estate 'B'.*—The death rate per mille for this estate has increased from 12.32 in 1927 to 42.29 in 1931, deaths among the adults predominating during the past three years, and being very high among the children over one year during 1931. As is mentioned in connection with Estate 'A' the increase is compatible with the increase in malaria. A death rate per mille of 42.29 is alarming, being considerably higher than the average for the province and shows that very heavy losses are being sustained by the estate, a matter which calls for remedy.

(b) *Infantile mortality.*

*Estate 'A'.*—The infantile mortality figures are about the average for India as a whole (*vide* Appendices I and II), and while there may be some losses through malaria, this does not appear to be a separate problem. Some decrease in the infantile mortality may be anticipated when anti-larval and other measures being to prove effective. Undoubtedly one of the causes of infantile mortality attributable to malaria is the reduced resistance and stamina of the infected mothers, who are not able to nourish their infants properly during their early existence.

*Estate 'B'.*—Considering the amount of malaria and other disease on this estate, the infantile and child mortality is surprisingly low, so much so in fact that special pains were taken during 1931 to be sure that all deaths were reported. Deaths among children rose from four in 1930 to twelve in 1931, whereas infant deaths in the same years were three and four respectively. The infantile mortality rate in 1927 being *nil* may be attributed to the small number of children born during that year.

(c) *Birth rate per mille.*

*Estate 'A'.*—The birth rate for the five years prior to 1931 was practically stationary, but in that year it came down to 26 63 per mille. The increase in malaria may have something to do with this slight reduction, but probably not as much as has the preponderance of males among the short-time labourers, which has considerably altered the ratio of males to females, there being 468 males to 312 females in 1926, as compared with 611 males to 345 females in 1931.

Births exceeded deaths by 76 during the six years under review.

*Estate 'B'.*—The birth rate on this estate has been anything but steady, suggesting some hidden cause for the fluctuations. The average for the five years is 28 65 per mille.

Total births exceeded deaths by 28 during the five years under review.

(d) *Sickness statistics.\**

*Estate 'A'.*—In a study of the first two columns of the sickness statistics (Appendices V and VI) and also of Graph 1, it will be seen that the curves for the cases admitted as 'Malaria' and the remaining cases under 'All diseases' follow one another practically for the entire six years. Those cases diagnosed 'Malaria' were approximately 50 per cent of the total. That the curves follow one another so constantly throughout the period suggests that a considerable amount of the balance of disease other than malaria is due to lowered resistance and is probably secondary to that disease. Therefore, provided successful anti-malarial work can be instituted, the total amount of

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\* Owing to the different methods employed on the two estates, it has been impossible to draw up statistics, and consequently graphs, in the same order.

sickness should be reduced in the same ratio as the reduction in the malaria incidence.

During the period, the increase in malaria admissions has been 52 per cent, which is out of proportion to that of the population, which has only increased 15 per cent. The increase is however in direct ratio to the increase of the new coolie population, composed largely of non-immunes.

In 1926 and 1927 admissions for malaria were responsible for 1,404 and 1,443 cases respectively, but in 1928 the figure increased to 1,775, in 1929 to 2,219, and in 1930 to 2,247, followed by a slight reduction in 1931 to 2,134, in spite of the fact that during this year all anti-malarial spraying and prophylactic quinine were stopped. Records previous to 1926 not being available, we can only assume that the figure of about 1,400 admissions represented the average amount of malaria prior to the year 1928, when that disease started to increase, as is evident from the graphs presented.

This increase corresponds with the increased recruitment of both permanent and short-term coolies, as also with the period when jungle clearing was instituted (1928), both as an anti-malarial measure and to clear certain land for the laying out of new lines and the extension of old ones. The possible effect of these two factors is mentioned elsewhere.

Table A gives the rate per mille of the pertinent figures, viz. :—

TABLE A.

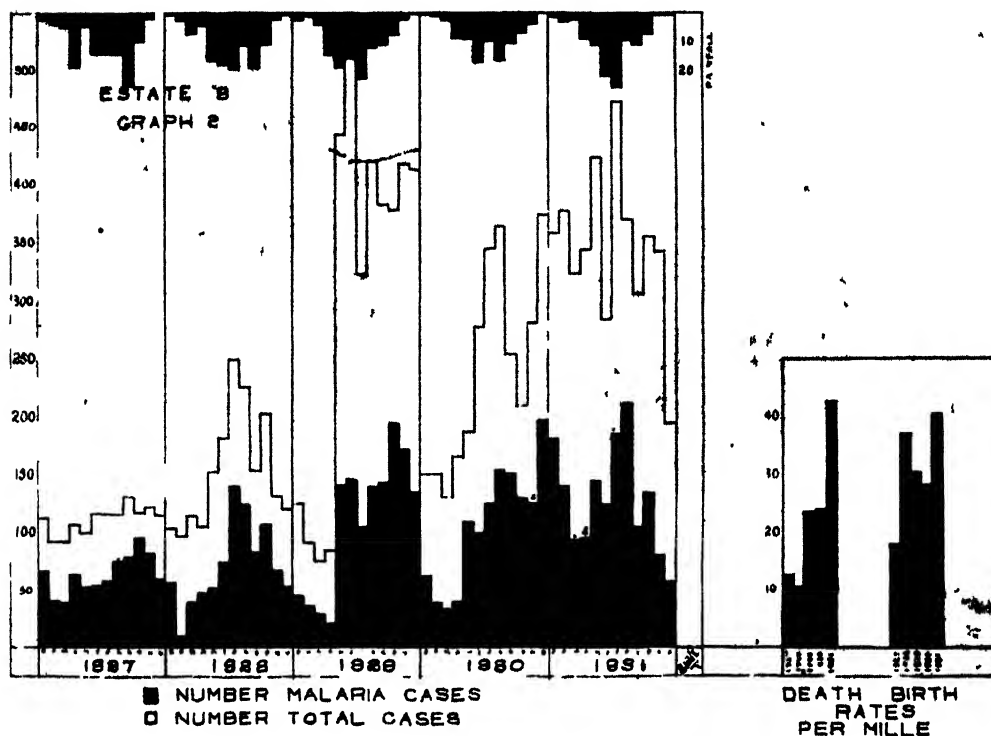
Year.	Popu-lation.	MALARIA ADMISSIONS.		TOTAL SICK ADMISSIONS.		TOTAL NUMBER DAYS SICKNESS.	
		Number.	Rate per mille.	Number.	Rate per mille.	Number.	Rate per mille.
1926	1,387	1404	1,012	2655	1,914	7974	5,749
1927	1,334	1443	1,081	2493	1,868	7806	5,851
1928	1,367	1775	1,298	2988	2,186	10198	7,460
1929	1,408	2219	1,576	3425	2,432	12502	8,879
1930	1,538	2247	1,461	3394	2,206	12020	7,815
1931	1,577	2134	1,353	3260	2,067	12383	7,788

This table gives a true picture of the increase of malaria proportionately with the increase in population, and shows that there has been an increase in the incidence of that disease of 30 per cent in 1931 as compared to 1926; the 1931 figure being lower than that of 1929, when the increase amounted to 50 per cent. It also shows that the increase in malaria bears a relation to the total sick admissions and also to the total number of days of sickness, all of which point to malaria being responsible for the greater bulk of sickness for the entire period.



*Estate 'B'.*—In studying the first two columns of sickness statistics (Appendices VIII and IX) and Graph 2, it will be seen that although the increase from all sick has a relationship to that of malaria, it does not follow in the same ratio as it did in connection with Estate 'A'. This feature may be due to the fact that malignant tertian malaria predominates on Estate 'B'. That malignant tertian presents a more varied symptom-complex than does the benign tertian is well known, and the findings would suggest that many

GRAPH 2.



cases which have been diagnosed and recorded as some other disease were, in reality, malignant tertian malaria. This statement is substantiated by the high spleen rate among the children, and the high malignant tertian parasite rate of the slides examined from this estate. If this be true, then the incidence of malaria as shown on the graph is considerably lower than it should be.

In a further examination of Graph 2, both 1927 and 1928 show that conditions, at that time, were approximately the same as those on the lower Estate 'A'; i.e., malaria representing about 50 per cent of all sickness. After 1928, however, when jungle clearing was instituted as a measure to combat

'blister blight', a disease of the tea plant, malaria was increased by nearly 80 per cent in the following year, and the total for all diseases by nearly 100 per cent, which rise has remained nearly constant during the last three years.

When we come to examine the rate per mille (Table B), we find that the conditions are actually worse than they are depicted on the graph, and as these figures show the position according to the population, they can be considered as the actual.

TABLE B.

Year.	Popu-lation.	MALARIA ADMISSIONS.		TOTAL SICK ADMISSIONS.		TOTAL NUMBER DAYS SICKNESS.	
		Number.	Rate per mille.	Number.	Rate per mille.	Number.	Rate per mille.
1927	893	754	844	1309	1,466	4993	5,591
1928	948	855	903	1840	1,941	5789	6,106
1929	929	1307	1,407	3670	3,950	7064	7,604
1930	1,048	1263	1,205	2897	2,812	9695	9,251
1931	922	1549	1,680	4565	4,515	13138	14,249

From this table it is readily seen that the increase in malaria since 1927 amounts to practically 100 per cent, rising from 844 per mille to 1,680 per mille, the total sick admissions rising from 1,466 per mille to 4,515, being an increase of over 200 per cent, while practically the same 200 per cent is the increase for total number of days of sickness. Further, this table shows the very definite rise in both the malaria and total sick admissions in the years following cutting of the jungle.

(e) *Labour losses.*

*Estate 'A'.*—In order to show the losses of labour occasioned by sickness, and the results of undermining of constitution by malaria, two tables must be given, viz. :—

TABLE C.

*Sickness : Labour loss in days, 1926–1931 inclusive.*

Year.				Malaria.	All diseases.
1926	..	..	..	2,840	7,974
1927	..	..	..	3,109	7,806
1928	..	..	..	3,804	10,198
1929	..	..	..	4,675	12,502
1930	..	..	..	5,013	12,020
1931	..	..	..	5,387	12,383
TOTALS				24,828	62,883
Average	..	..	..	4,138	10,480

TABLE D.

*Labour statistics : combined.*

Year.	Average monthly labourers on books.	Available days labour.*	Actual days labour.	Percentage, labour efficiency.
1926 ..	915	274,500	159,538	58'12
1927 ..	759	227,700	137,377	60'33
1928 ..	863	258,900	147,091	56'81
1929 ..	879	263,700	158,532	60'12
1930 ..	901	270,300	183,209	67'77
1931 ..	1,061	318,300	214,396	67'35
TOTALS ..	..	1,613,400	1,000,143	
Average ..	..	268,900	166,690	

\* 'Available days labour' taken at 300 working days per year per working coolie on the books.

In Table C, the losses directly due to malaria average 4,138 days and from all diseases 10,480. Estimating that 25 per cent of the balance of sickness other than malaria is primarily brought about through lowered resistance due to that disease (and this is probably a low estimate), we must add another 1,585 days to the 4,138 due directly to malaria, making the total 5,723 days loss due directly and indirectly to malaria.

As the percentage of efficiency only averages about 60 throughout the entire period under review, and as a great many coolies when ill do not report to the hospital for treatment, we must evaluate this loss also, which we take at 5 per cent of the total absentees. The difference between the 'Average available days labour' of 268,900, and the 'Average actual days labour' of 166,690, represents those labourers not actually at work from all causes, the difference being 102,210. Five per cent, which we will consider the proportion of days lost, is 5,110, and represents incapacitation of absentees due directly and indirectly to malaria. This figure is probably low, for many adults absent themselves from work in order to tend their children who are suffering from malaria, whilst others who contract malaria do not report for treatment.

Rice (1931), in his Doears investigation, when estimating losses among coolies actually at work, says, 'It can readily be seen that coolies constantly being subjected to malarial infections, and to bouts of malaria as well as other

infections, cannot, as a whole, be anything like 100 per cent efficient, and it is evident that this lack of efficiency must show up in the quality and quantity of their work, whatever it is'. He estimated a minimum of 10 per cent reduction of efficiency for all diseases, and, in the case of the Dooars, 6 per cent through primary and secondary malaria. As has been shown previously, the average loss from all diseases is 10,480 days and the direct and indirect losses from malaria 5,723 days which is slightly more than 50 per cent of all disease. We shall use this ratio in relation to the loss of efficiency of actual working coolies, or, in other words, take 5 per cent as representing the loss sustained. Therefore, the 'Average days actual labour' being 166,690, a 5 per cent efficiency loss due to malaria gives us a total of 8,334 days labour loss to this account.

With regard to the loss occasioned by deaths, there were 39 during the six years directly due to malaria, or an average of 6.5 per annum. Estimating that of the balance of 128 deaths, 25 per cent were indirectly due to malaria through the undermining of constitution, this gives us a further 31.75 deaths, or 5.3 per annum, making a total of 11.8 deaths per annum due to malaria.

Summary of the losses incurred gives us the following averages for the period 1926-1931 inclusive :—

1. Average number of deaths due primarily or secondarily  
to malaria            ..            ..            ..            .. 11.8
  
2. Average number of days registered sick due to malaria,  
i.e., 4,138 plus 25 per cent of balance of sick  
(1,585) or            ..            ..            ..            .. 5,723
  
3. Estimated average number of absentees due to malaria,  
i.e., 5 per cent of labourers not actually at work  
from all causes (5 per cent of 102,210), or            .. 5,110
  
4. Estimated average days efficiency loss due to malaria  
(working coolies only), i.e., 5 per cent of 166,690,  
or            ..            ..            ..            .. 8,334

Total number of days labour lost through malaria .. 19,167

*Estate 'B'.*—We follow as nearly as possible the same method of estimating labour losses as was done in respect of Estate 'A'.

TABLE E.

*Sickness : Labour loss in days 1927-1931 inclusive.*

Year.				Malaria admissions, actual.	Malaria days loss (estimated)*	All diseases, days loss, actual.
1927	..	..	..	754	2,262	4,993
1928	..	..	..	856	2,568	5 789
1929	..	..	..	1,307	3,921	7,064
1930	..	..	..	1,263	3,789	9,695
1931	..	..	..	1,549	4,647	13,138
TOTALS ..				5,729	17,187	40,679
Average	..	..	..	....	3,437	8,135

\* Figures not being available for the total days labour loss due to malaria, we can only estimate the average days incapacitation from the number of malaria admissions. In studying the sick registers available, the average length of treatment is approximately three days, hence we use this figure in estimating losses.

TABLE F.

*Labour statistics : combined.*

Year.	Average monthly labourers on books.	Available days labour.*	Actual days labour.	Percentage labour efficiency.
1927 ..	497'41	149,233	102,726	68'84
1928 ..	493'51	148,053	107,949	72'91
1929 ..	513'51	154,053	110,550	71'76
1930 ..	558'75	167,625	118,524	70'71
1931 ..	569'75	170,925	113,199	66'23
TOTALS ..	....	789,889	552,948	
Average ..	....	157,978	110,589	

\* 'Available days labour' taken at 300 working days per year per working coolie on the books.

We will consider the losses on the same scale and principle as those worked out for Estate 'A' and therefore give here only the summary :—

1. Average number of deaths due primarily or secondarily to malaria      ..      ..      ..      9.5
  2. Average number of days registered sick due to malaria, i.e., 3,437 plus 25 per cent of balance of sick (1,174), or      ..      ..      ..      4,611
  3. Estimated average number of absentees due to malaria, i.e., 5 per cent of labourers not actually at work from all causes : (5 per cent of 47,389), or      ..      2,369
  4. Estimated average days efficiency loss due to malaria (working coolies only), i.e., 5 per cent of 110,589, or      ..      ..      ..      5,529
- Total days labour lost through malaria      ..      12,509

(f) *Finance.*

*Estate 'A'.*—In order to arrive at a sum which can be spent economically on an anti-malarial scheme, both the capital value of the coolie and also his daily value to the estate must be estimated.

From the figures available, we have not been enabled to arrive at any definite sum as to the cost of recruiting, but must take, for determining the value of labour losses, an estimated figure. In the Dooars, the value to the estate of the settled working coolie was estimated at Rs. 400 (Rice, 1931). For Assam Sir William McKercher, late Chairman, Assam Branch Indian Tea Association, who interested himself in this matter, worked out the figure as in the close neighbourhood of Rs. 500. However, for sake of comparison with the paper by Rice previously mentioned, we here use the estimated evaluation of Rs. 400 per working coolie, and thus, the average number of working coolies on the books of this estate being 893 for 1926 to 1931 inclusive, the capitalized value of the labour force to the estate becomes Rs. 3,57,200.

The value, per acre, of this Company's tea area is approximately £75, therefore this particular estate being of 714 acres has a total valuation of £53,550. As the profit (not dividend) for the years 1926 to 1931 inclusive was approximately £55,641, this gives an average of £11,128 per annum, and represents approximately 20 per cent per annum on the capital involved.

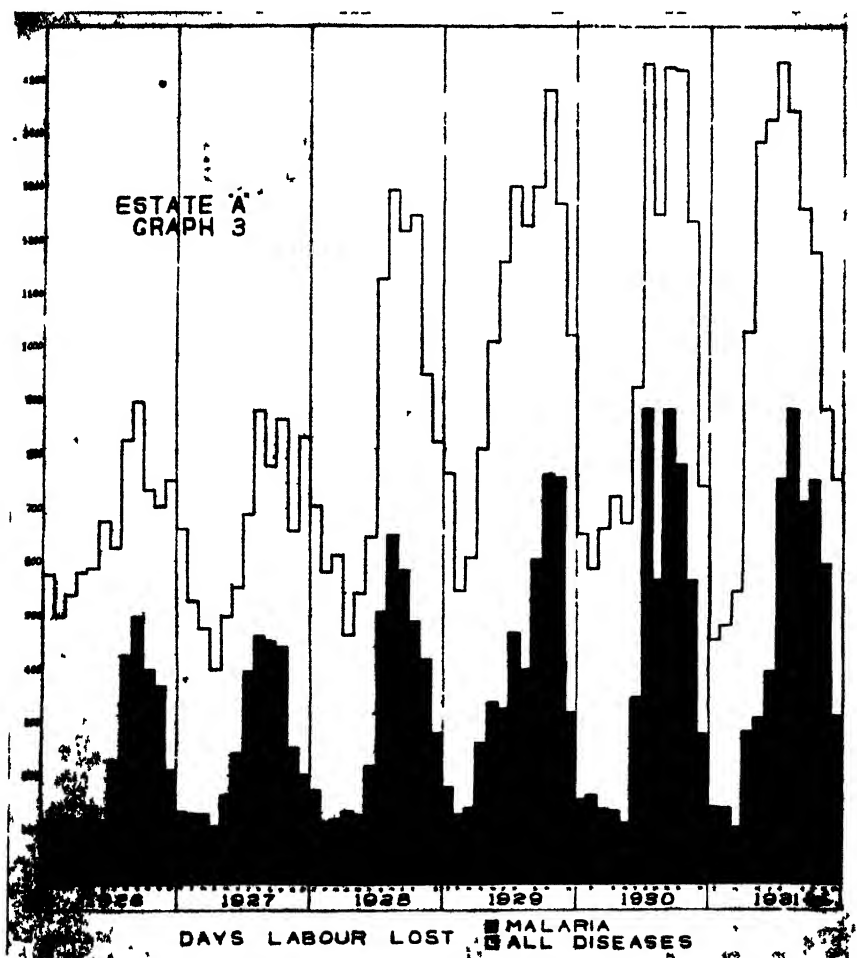
The average profit, Rs. 1,48,336 (£11,128 per annum = Rs. 1,48,336 at 1s. 6d. per rupee), divided by the average total actual labourers at work, which is 166,690 (Table D), gives the profit derived from each coolie's labour per day and is As. 14-3.

As the pertinent figures in connection with pay of the coolie are not available, we estimate this figure (as in the Dooars) to be As. 8 per day per coolie.

The yearly costs of coolie housing, repairs to housing, of water supplies, of hospital and medical expenses, of recruiting expenses, of the various bonuses and other perquisites given to coolies, can be estimated as approximately As. 6 per day per coolie.

The average value of each coolie's work, therefore, can be evaluated at approximately Re. 1-12-3 per day. This figure is slightly higher than that

GRAPH 3.



for the Dooars Estates (Rice, 1931), which was only Re. 1-8, but this can be accounted for partially by the higher price, per pound, obtained for the tea manufactured on Assam Estates.

In evaluating the annual losses, we now have the basic figures of Rs. 400 for the settled coolie, and the estimated Re. 1-12-3 as the value of his labour.

Taking the figure Rs. 200 to represent the average loss by deaths of both children and adults together, and deaths due to malaria averaging 11·8, the total average loss through deaths becomes Rs. 2,360 per annum.

The average total days labour lost through malaria being 19,167, and the value of the coolie's labour being Re. 1-12-3 per day, this then gives a total loss to the estate of Rs. 33,841 per annum, which, when added to the Rs. 2,360 loss from deaths, gives a total average loss of Rs. 36,201 per annum, which is over 10 per cent per annum on the capitalized value of the entire working force.

The total annual losses being Rs. 36,201, then working on the 'Eight Years Purchase of Profit Valuation' basis, the capitalized value of the loss caused by malaria to this estate becomes Rs. 2,89,608, i.e., eight times Rs. 36,201.

Aiming at a 50 per cent reduction in the malaria incidence, and being confident of a minimum of 25 per cent reduction, we take the latter figure as a definite goal, thereby reducing the capitalized value of malaria for our present purpose to Rs. 72,402. We now suggest that the interest on this sum at 5 per cent, or Rs. 3,620, is the amount which can justifiably be expended per annum on reducing the incidence of malaria on Estate 'A'.

*Estate 'B'.*—As in the 'Labour losses', the finance figures are worked out on the same scale and principle as those for Estate 'A', and we give only a summary of the figures, viz. :—

1. Capital value of coolie to the estate .. ..	Rs. 400
2. Capital value of labour force (average working coolies on books 526·58 at Rs. 400) ..	Rs. 2,10,632
3. Value tea area of estate (400 acres at £75 per acre)	£30,000-0-0
4. Approximate profit, 1926-1930 inclusive ..	£24,050-0-0
5. Average annual profit, 1926-1930 inclusive ..	£4,810-0-0
6. Average annual profit, 1926-1930 inclusive (at 1s. 6d. per rupee) .. ..	Rs. 64,117
7. Profit derived from each coolie's work per day, (average annual profit, Rs. 64,117, divided by average total actual labour at work, 110,589, see Table F) .. ..	As. 9-3
8. Average pay (estimated at As. 8) .. ..	As. 8
9. Average cost perquisites, etc. (estimated) ..	As. 6
10. Average daily value coolie's labour .. ..	Re. 1-7-3



The actual losses in rupees are as follows :—

1. Losses through deaths due to malaria (average deaths 9.5 at Rs. 200)	..	..	1,900	0	0
2. Labour loss due to malaria (12,509 days at Re. 1-7-3)	..	..	18,177	2	0
3. Total losses due to malaria	..	..	20,077	2	0
4. Capitalized value of loss caused by malaria to estate (eight years purchase of profit valuation)			1,60,617	0	0
5. Estimated 25 per cent reduction in malaria incidence, then capitalized value becomes	..	..	10,154	4	0
6. Annual sum justifiably expendable in reduction of malaria (5 per cent of Rs. 40,154-4-0)	..	..	2,007	11	0

(g) Recruiting.

*Estate 'A'.*—The following table shows the number of 'permanent' and 'short-term' *adult* coolies recruited during six years :—

TABLE G.

Year.				Permanent.	Short-term.	Total.
1926	..	..	..	25	<i>Nil</i>	25
1927	..	..	..	113	49	162
1928	..	..	..	163	6	169
1929	..	..	..	137	163	300
1930	..	..	..	109	129	238
1931	..	..	..	117	162	279
TOTALS				664	509	1,173

During the past six years 664 permanent and 509 short-term adult coolies have been imported, but in spite of this fact, the total population has only increased 190, i.e., from 1,387 in 1926 to 1,577 in 1931. During this same period, births on the estate have been 66 in number more than deaths. As the 162 'short-term' coolies at present on the estate are included in the population figure for 1931, and as there have been 66 more births than deaths during the six years, we can safely say that the permanent population is practically the same in number to-day as it was in 1926, although 664 permanent coolies have been recruited. There has, however, been an increase in the average daily number of labourers available from 915 in 1926 to 1,061 in 1931.

Short-term coolies have been coming to the estate in greater numbers during the past three years, and this would appear to be a fairly firmly established method of obtaining labour for this garden. These coolies come to the

garden for various contract periods ranging from nine months to two years, during which time they put in as many working days as possible. Owing to this fact, and also that they are not accustomed to hospital treatment, they rarely present themselves for such treatment when they are ill, unless they are made to. The larger proportion of these coolies come from areas in which there is relatively little malaria, and their immunity to this disease is therefore low when compared with that of the locally-born coolie. Under these circumstances short-term coolies must increase the malaria ratio where the malaria infectivity rate is high, and at the same time, must also increase the death rate per mille, as they often do not present themselves for treatment until they are *in extremis*; this latter factor probably plays an important part in the increase of the death rate from 10.81 in 1926 to 28.53 in 1931.

There would appear to be approximately a 10 per cent loss of labouring coolies per annum apart from the losses sustained by deaths, or, in other words, the entire labour force of about 1,000 coolies is being replaced by recruiting every ten years. This does not necessarily mean that all of the coolies are being replaced, as the greater proportion of those leaving the estates are actually newly-recruited labourers. This very heavy turnover of labour has the effect of keeping the malaria index higher than it would be with only immunized labourers, such as those from a hyperendemic area, and it undoubtedly increases the chances of infection and re-infection of the local-born coolie residents. There is, as has been previously shown in the labour statistics, an annual loss of about 35 per cent efficiency of the labour present, and considering that the short-term coolies work as many days as possible, this means that among the old-established coolies the percentage of efficiency must be less than 65; therefore an excess of 35 per cent of labour must be carried, or made up by recruiting, at considerable cost to the garden. Much of this loss has been shown to be due to malaria; hence, as the results of malaria control measures begin to be felt, fewer recruits will be necessary to balance losses due to sickness. The consequent reduction in the number of susceptible non-immunes will lead in addition to a further decrease in the incidence of malaria.

*Estate 'B'.*—Only permanent coolies have been recruited for this estate, the figures for which are given as follows :—

TABLE H.

Year.	1927.	1928.	1929.	1930.	1931.	Total.
Recruits.	36	62	63	71	72	304

Births numbered 134 and deaths 106 for the period 1927–1931. The population increased from 893 in 1927 to 922 in 1931, an increase of 29 only, in spite of the fact that there were 28 more births than deaths and 304 coolies

had been recruited. The losses in labour are said to be principally among the newly-recruited coolies.

The percentage of efficiency in 1927 was 68.84, rising to 72.91 in 1928 and then gradually falling in ratio to the increase in sickness to 66.23 per cent in 1931. This shows a loss of over 30 per cent which, in the case of this estate, must be compensated for by recruitment alone. The extra expense in carrying this great number of excess labourers, in recruiting, housing, etc., might be reduced by increasing the efficiency of the present existing labour force through treatment and anti-malarial measures, when, with the reduced recruiting, the same reduction of sickness mentioned in connection with Estate 'A' would take place.

#### IV. FACTORS INFLUENCING THE MALARIA RATE.

##### (a) *Clearing jungle.*

*Estate 'A'.*—Prior to 1928, a comparatively heavy strip of jungle was present between the then main lines and the boundary of this estate, towards what is now Line 21. Since that time this jungle has been cut down from year to year, leaving undulating land which is low-lying and liable to flood when the K— stream backs up. These floods are probably helpful in washing out breeding areas, but between floods much clear water suitable for *A. minimus* is present. In drains leading from the main lines in this area we have found larvæ of *A. minimus*. As these drains were prior to 1928, covered with jungle, the cutting down of this has made them most suitable for the breeding of this species. This supposition is supported by the increase in malaria as is shown on Graph 1 of this estate.

*Estate 'B'.*—The reasons given for clearing jungle on this estate are that it obstructs the view of the tea and that it encourages the spread of 'blister blight'. As we suspect this clearing gravely to affect the malaria incidence, we add the following details :—

Blister blight, a disease of the tea bush, caused much loss of crop in 1927, when the Scientific Officer for the Company proposed clearing all jungle from the edges of the tea area. As the tea-growing land was intersected by streams covered by jungle, it was ordered that this be cleared, which work was carried out in 1928. With our present knowledge, through this survey, we realize that *A. minimus* is the principal vector of malaria on this estate, and that this particular species does not propagate in streams covered with heavy jungle (Ramsay, 1930), but does so when sunlight is admitted. The supposition is that by the cutting of the jungle, conditions have been made more favourable for *A. minimus*, and this supposition is adequately substantiated by the very great increase in malaria in 1929, 1930 and 1931 as shown in the accompanying Graph 2 for this estate.

Upon taking up the question of blister blight with the Scientific Department, Indian Tea Association, Tocklai, we have been informed that this is a

disease affecting two plants only, namely, the rhododendron and the tea bush, and is not actually transmitted through any jungle plant. The only danger from such jungle is when it actually shades the tea bush, which shade, when present, is conducive to the spread of the disease. As the jungle which affects us particularly, in relation to anti-malarial work, is on the low stream edges, this could not, according to the above information, be the cause of any increase in blister blight. Clearing it has however obviously been the cause of the increase in malaria-carrying anophelines and therefore the great increase in malaria since 1928. We therefore strongly recommend that none of the jungle growing along stream edges be cut under any circumstances, but that it should, on the other hand, be encouraged to grow and even cultivated and planted if found to be necessary. By this we do not mean that it is necessary to allow jungle to grow into the tea, as practically all of the streams and ravines are sufficiently deep to allow jungle to be cut back from the tea for at least 8 to 10 feet without interference with the degree of growth we consider necessary.

#### (b) *Recruiting.*

The effect of recruiting on the incidence has been discussed in the previous section and its importance stressed.

It has been shown by Ross (1911) that the malariousness of a stable population tends towards a fixed level. The importing of labour has a very profound effect upon this malaria level on any estate, and may increase it to epidemic proportions by the bringing-together of susceptible people from healthy areas with the partially immune from malarious areas. These non-immune arrivals, being highly susceptible to malaria infections and therefore to rapid multiplication of malaria parasites, act when infected as a reservoir of infection, as do children, the younger of whom are non-immune. Thus they tend, probably, greatly to increase the number of infected mosquitoes with a subsequent re-infection of the entire labour force.

### V. RECOMMENDATIONS.

#### (a) *General.*

As the malaria phenomenon is not a static but a constantly changing one, we do not feel justified in recommending anything more than the minimum in the way of anti-malarial programme until the problem has been further studied. A system of regular inspection is essential in any control work, and we recommend that a 'Malaria Babú', such as is employed on many estates, be appointed to inspect breeding places, identify species, record infectivity and parasite rates, and undertake such anti-malarial work as has been recommended, or that the Medical Officer may in future consider necessary. Such a Babu can be obtained for a salary of about Rs. 30 a month, and he should commence work immediately. In the interests of economy and efficiency, careful entomological supervision is required, so that measures be limited to

areas where vectors are actually found. To this end a good Malaria Babu will more than pay for his keep, for he will economize time and oil, and ensure oil being applied only in the right places and at the right times.

(b) *Detailed.*

*Estate 'A'.*—It should not be very difficult to control malaria in the main lines. The only places where *A. minimus* has been shown breeding are in the main drain, about half-way between hospital and *pathar* into which it empties. Other parts of this drain, and its tributary drains, have been searched on many occasions without finding any of this species. We recommend that bags of cotton waste soaked in waste oil be anchored on each side of the main drain at intervals of 100 yards, and that these be changed at weekly intervals; that the malaria inspector survey all parts of this main drain at weekly intervals and that if any *A. minimus* larvæ be found, such situations be sprayed with oil.

There is seldom, if ever, a reversed flow in this drain, so that a small amount of oil, applied near the hospital end, should keep a large section of the drain free from breeding.

Water on the rough land should also be regularly searched for breeding, and oiled as found necessary. As the jungle over this rough area has now been partially cut, we think the best policy for the Company would be gradually to clear and level completely so that at some future date it might be available as an extension for a line site. Partial jungle would be more dangerous than none at all, as it will hinder the spread of oil and prevent the finding of breeding areas. On this land, so near the lines, dense shade could never be re-established because of cattle trespass and fire-wood cutting by the coolies. As levelling is expensive, and over such a large area would entail a programme of many years, we think that oiling, and possibly burning off each winter, would be the most economical policy to pursue.

Regarding section drains, we recommend that ferns and other overhanging vegetation be disturbed as little as possible in the process of cleaning, so that the dense shade factor may operate.

Line 21 will be benefited considerably by any work done to the main lines. The stream which adjoins it at the southern end is its great source of trouble. This is probably not so dangerous in the middle of the rainy season, when the rise and fall of many feet of water flushes out considerable numbers of larvæ. We think it would be advisable to inspect the portion of it adjacent to the lines weekly from March to November, and oil as necessary. There are numbers of borrow-pits in these lines which it would be advantageous to connect up with each other and eventually to fill in. Additions of cattle manure weekly to these small collections of water would hinder breeding of *A. minimus*, and would also help in filling in these depressions.

Drains along the boundary fence, as stated elsewhere, are becoming covered with dense ferns, which growth should not be interfered with.

Lines 15, 16 and 17 are on the edge of the *pathar* and do not appear as unhealthy as the others. As they lie between the *pathar* and tea area, no work other than that on drains can be attempted. Work around the main lines should benefit these also.

Line 20, where the short-term coolies are housed, is the most malarious of all. Many of the new arrivals are non-immune and quickly succumb to infection. The adjacent plots of land belong to eight different people, apart from Plot No. 150, which is Government land. It is evident that anti-larval measures are not easy under such conditions. Only a small portion of this land is cultivated and remainder is low-lying. The only recommendation we make for this line is that the breeding areas immediately surrounding it be oiled as balance of material from main lines allow. In this way, as much as possible will be accomplished on the estimates submitted.

We think that no new houses should be built on this site, and, if possible at some future time, the present ones should be shifted to a healthier one, where anti-malarial measures are possible.

*Estate 'B'.*—This garden is so much isolated (about four miles from nearest village) and chances of heavy re-infection from outside sources, for that reason, are so much less, that it rather lends itself to control. A partial solution of the problem is possible as it is simplified by the fact that bungalow, factory and main lines are so close together. The perennial streams are the chief danger here, as evidenced by the finding in them of the larvæ of many dangerous species of anopheline mosquitoes. From the point of view of biological control, much of this water would not be difficult to cover completely with dense shade, if it were not cleared annually. In some places the banks are of rock or gravel and the task would be more difficult, e.g., at their terminal portions, which for a distance of about two hundred yards are ten to twenty feet wide when flooded. This flooding kills off much of the natural growth.

If jungle be not encouraged to shade these streams and ditches completely, they will require the application of oil or other larvicide weekly throughout the transmission season. As an alternative to oiling, in the terminal parts of the perennial streams, where dense growth is not possible through the character of the bed of the stream, the edges of the stream could be swept out thoroughly into the river twice weekly. As breeding places vary tremendously with height of water, constant inspection by a trained man is necessary.

As far as *hoolas* are concerned, there is a natural dense growth of *tarapat* in many of them and several seasons planting could completely cover many more. Line drains and irrigation ditches could be dealt with in a similar way. As all this work would require much labour and supervision, and entail a programme of several years to make effective, we do not think any scheme of planting should be embarked upon at present, but certainly jungle may be left to grow of its own accord in these situations.

There are three possible methods of control, namely, extermination of the anophelines, complete protection from bites or the sterilizing of the blood of

the population by the destruction of gametocytes. This last method, which is temporarily possible by drug administration, we think should be tried first, and if there follow a reasonable reduction in malaria, it can be continued as necessary in subsequent years. It will be worth while to see how long the population, when the principal carriers are treated and cured, will remain without re-infection, since if the period be considerable it will be the cheapest method of control for this estate. With such isolation the visits of the coolies to the neighbouring bazaar are few and almost all by day, and consequent infection picked up in this manner very little.

As it is only the sexual form of the parasite that can infect the mosquito, a thorough treatment of children, who are the main source of infection, would do much to reduce the malaria of this garden. Our aim would be, by a regular course of Plasmoquine, to break the vicious circle long enough for the infected generations of mosquitoes to die out. Re-infection then will become much less and the natural resistance of the individual, with aid of ordinary treatment with quinine, can be left to overcome any subsequent infection.

An advantage of systematic treatment of children is that it gives them a chance of better development from the start, instead of being almost overwhelmed with infection from birth, and it enables them to put up a better fight against infection when it does occur. An immunity developing more gradually would appear to be less costly to the individual and to the employer. We recommend a preliminary course of one half to one tablet Plasmoquine Simplex, i.e.,  $1/6$  to  $1/3$  grain, each day for eight consecutive days, and a second course as examination of the blood shows it to be necessary.

All adult cases presenting themselves to hospital with malaria should receive a minimum of 20 grains of quinine and  $1/3$  to  $2/3$  grain Plasmoquine Simplex for at least three days, chronic cases receiving eight days treatment. Dosage would be varied according to age, etc.

#### (c) *Estimated costs.*

*Estate 'A'.*—Actual breeding areas of *A. minimus* found around the main lines and bungalows of Estate 'A' were few, so that control here appears a sound business proposition. In addition to this any oiling done near the main lines will benefit, not only these and bungalows, but also Christian lines, Line 21 and, to a less degree, Lines 15, 16 and 17, since all are grouped within a few hundred yards of each other.

The costs for the main drain from the hospital to its termination (i.e., the only part in which any *A. minimus* larvæ have been found in the six months survey) would be extremely small. The drain is straight and flows always in one direction, so that oiling can be done automatically without leaving areas without a protective film, as would happen if there were corners or irregular margins. The method of using cotton waste soaked in oil, e.g., waste oil from the factory, is most economical and, except for changing once weekly, is automatic.

The cost for oiling the rough area, west and south-west of lines, depends entirely upon what proportion of it, from time to time, is found to be a source of breeding. Naturally, the female mosquitoes do not deposit eggs in exactly the same places every time. The whole area is, roughly, 20 acres in extent, of which, probably, it will be found necessary to oil a total of 2 acres per week. As it would be extremely costly to oil the entire area weekly, the most economical way of dealing with it will obviously be by regular weekly inspections for larvæ by the Malaria Babu, to ensure that the whole area be controlled. Two acres would require about 30 gallons of oil per week, or 900 gallons for the entire breeding season. This estimate will include possible spraying on the main drain, which passes through this area, i.e., if waste oil be insufficient to control breeding there.

As regards the recommendation that the K—— stream be given weekly treatments in certain periods of the year, it has been estimated that one gallon of oil will suffice for 250 yards by 1 foot, that is, roughly one hundred yards of both sides of a river or stream. As the stream involved bends away from the line in both directions, and only approaches it at one point, and that at one extremity of the line, we estimate that 10 gallons of oil, per application, will be sufficient to control any parts of it which are giving trouble. With twenty weekly applications, therefore, the total amount required would be 200 gallons.

There are several miles of drains affecting the malarial problem of all the lines, the principal drains of which are one lying between the Superintendent's bungalow and Line 20 running parallel with the main road at a distance of about 300 yards from the bungalow, and another arising behind the Superintendent's bungalow and running towards the K—— stream and parallel to Line 21 at about the same distance from bungalow. As vegetation covering much of these drains has been cleared, we must allow material to control at least one mile of them, until such time as dense shade can again be established. We estimate that the amount of oil required for this will be about 250 gallons.

We think that section drains, borrow-pits, pools and other collections of water may require an additional 250 gallons for the breeding season.

We estimate the total amount of oil that would be used in the season would be 40 casks of 40 gallons each, but as the transmission period might be prolonged, we have allowed an additional 10 casks in the budget. A 40-gallon cask of anti-malarial oil costs, at present, Rs. 17-8, plus freight, as supplied by the Assam Oil Company, Limited. It has excellent spreading qualities, high toxicity for larvæ, and burns grass well, so that after a few applications much less oil is required. It has been recommended by Ramsay and others. For the freight and local carriage, we have allowed Rs. 80.

Other expenditure is pay of a Malaria Inspector and of coolie labour. As the major part of the Inspector's time will be spent on Estate 'A', we recommend that the cost of 2/3rds of his salary, i.e., Rs. 20 per month, be borne by this estate, and the remaining 1/3rd by Estate 'B', as he will be required to attend the latter for blood examinations, etc., and as detailed by the Medical



Officer. We estimate that two coolies' labour would be sufficient to oil the area concerned, which will be mapped out so that they can readily cover the whole ground weekly.

Minor expenses, to include repairs to pumps, etc., are taken at Rs. 65. New pumps are not required at present.

In view of the foregoing details we suggest that the total appropriation of Rs. 1,500 be sanctioned, to be apportioned as follows :—

	Rs.
50 casks oil at Rs. 17-8 .. ..	875
Freight on above .. ..	80
2/3rds Malaria Inspector's salary for 1 year ..	240
Labour, 8 months at Rs. 15 for 2 men ..	240
Minor expenditure .. ..	65
Total ..	1,500

This expenditure on anti-malarial work is considerably less than that for the same purpose in many gardens in Upper Assam and the Surma Valley. It is slightly more than that spent in this estate yearly prior to 1931, before oiling and prophylactic quinine were stopped. Prophylactic quinine given in 10 grain doses, twice weekly, for a period of five months to the labour force of approximately 1,000 coolies, would cost between Rs. 1,300 and Rs. 1,500 per annum, and would not be nearly as effective as oil to that value applied under proper supervision.

*Estate 'B'.*—We do not consider that any expenditure for anti-malarial measures should be made at present, as jungle will cover most of the areas if allowed to grow. We therefore propose confining our attention to treatment, which obviously is necessary, as practically the whole population has been found infected. The cost of treatment as recommended we estimate at Rs. 351 for 5,000 tablets Plasmoquine Simplex, grain 1/3.

One coolie will be required for approximately three days a week to sweep out the lower reaches of the streams.

A total estimate of Rs. 500 should be sufficient to cover every contingency for this estate.

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## APPENDIX I.

## Estate 'A'.

*Vital statistics, 1926 to 1931 inclusive.*

Year.	Popula- tion.	DEATHS.				Births.	Infantile mortality per mille.	Death rate per mille.	Birth rate per mille.
		Infants.	Children	Adults.	Totals.				
1926	1,387	8	4	9	21	42	190'47	10'81	30'28
1927	1,334	9	7	5	21	45	214'00	11'24	33'73
1928	1,367	4	13	14	31	42	95'23	20'48	30'72
1929	1,408	5	8	21	34	44	113'63	21'31	31'25
1930	1,538	9	12	19	40	50	180'00	22'11	32'51
1931	1,577	9	22	21	52	42	214'28	28'53	26'63

## APPENDIX II.

## Estate 'B'.

*Vital statistics, 1927 to 1931 inclusive.*

Year.	Popula- tion.	DEATHS.				Births.	Infantile mortality per mille.	Death rate per mille.	Birth rate per mille.
		Infants.	Children	Adults.	Totals.				
1927	893	0	3	7	10	15	0'00	12'32	17'92
1928	948	2	2	6	10	35	57'14	10'55	36'92
1929	929	4	5	13	22	28	142'86	23'68	30'14
1930	1,048	3	4	18	25	19	157'89	23'85	18'13
1931	922	4	12	23	39	37	108'11	42'29	40'13

## APPENDIX III.

## Estate 'A'.

*Actual labour on books (working coolies) from January 1926 to December 1931.*

	1926.			1927.			1928.		
	M.	W.	C.	M.	W.	C.	M.	W.	C.
January ..	432	351	143	320	167	121	387	275	139
February ..	431	350	143	326	266	121	390	277	135
March ..	436	354	165	337	272	112	415	279	144
April ..	443	354	166	342	278	117	425	283	142
May ..	365	292	132	350	268	121	439	286	143
June ..	368	296	123	367	268	124	465	295	145
July ..	368	296	122	392	278	113	449	306	132
August ..	367	297	122	377	272	142	455	307	133
September ..	368	295	123	379	280	146	456	307	133
October ..	370	298	124	471	284	146	436	306	133
November ..	350	298	126	384	286	147	439	307	133
December ..	320	267	121	396	287	148	418	305	133
TOTALS ..	5,618	3,748	1,610	4,441	3,106	1,558	5,174	3,533	1,645
YEARLY GRAND TOTAL.		10,976			9,105			10,352	
MONTHLY AVERAGE.		915			759			863	

	1929.			1930.			1931.		
	M.	W.	C.	M.	W.	C.	M.	W.	C.
January ..	370	268	130	434	283	130	498	308	95
February ..	371	268	131	438	288	137	522	319	97
March ..	396	280	131	450	290	138	594	344	98
April ..	427	290	128	465	302	140	662	364	105
May ..	471	296	131	483	281	104	658	368	111
June ..	493	322	138	513	304	103	654	365	111
July ..	506	322	138	521	308	97	659	357	110
August ..	500	338	141	546	318	96	646	357	112
September ..	502	338	140	546	316	102	644	353	108
October ..	464	291	133	497	299	102	612	343	109
November ..	440	279	132	495	302	99	612	342	108
December ..	436	280	133	490	301	99	570	317	100
TOTALS ..	5,376	3,572	1,606	5,878	3,592	1,347	7,331	4,137	1,264
YEARLY GRAND TOTAL.		10,554			10,817			12,732	
MONTHLY AVERAGE.		879			901			1,061	

Monthly average for the period January 1926 to December 1931. 893.

## APPENDIX IV.

## Estate 'A'.

Statistics : Actual labour at work from January 1926 to December 1931.

	1926.			1927.			1928.		
	M.	W.	C.	M.	W.	C.	M.	W.	C.
January ..	6,892	5,436	2,224	7,284	5,352	2,019	7,453	4,468	1,967
February ..	6,201	4,891	2,171	7,149	4,972	2,002	6,727	4,175	1,565
March ..	6,159	4,821	2,138	4,811	3,686	1,947	7,120	4,252	2,275
April ..	6,983	5,369	2,311	5,509	4,186	2,058	6,645	4,594	1,679
May ..	7,024	4,964	2,063	5,397	4,405	1,796	6,347	4,386	1,664
June ..	6,958	4,980	2,105	5,272	4,462	1,405	5,854	4,792	1,997
July ..	6,252	4,654	2,243	4,983	4,302	1,293	6,170	4,993	2,006
August ..	6,248	4,989	2,198	5,442	4,179	1,185	5,820	4,855	1,964
September ..	6,360	4,872	2,158	5,747	4,059	1,246	5,702	4,705	2,005
October ..	6,302	4,759	2,005	5,636	3,987	1,199	5,474	4,463	1,884
November ..	5,072	4,248	1,957	5,471	3,778	1,105	4,118	4,184	1,872
December ..	5,297	4,132	2,102	5,229	3,765	1,055	4,217	2,974	1,635
TOTALS ..	75,748	58,115	25,675	67,928	51,133	18,316	71,647	52,931	22,513
YEARLY GRAND TOTAL.		159,538			137,377			147,091	

	1929.			1930.			1931.		
	M.	W.	C.	M.	W.	C.	M.	W.	C.
January ..	6,909	4,389	2,462	8,163	4,649	1,993	9,548	5,138	1,627
February ..	6,892	4,198	2,563	7,196	4,237	1,995	9,692	5,182	1,511
March ..	6,901	4,046	2,242	7,530	4,148	1,312	10,426	5,128	1,472
April ..	6,952	3,736	2,237	8,672	4,499	2,160	12,131	5,760	1,883
May ..	6,830	3,716	2,129	9,646	4,736	1,964	11,712	5,298	1,715
June ..	6,444	3,613	2,055	9,456	4,388	1,800	12,079	5,607	1,762
July ..	6,581	4,183	1,927	9,699	4,485	1,761	9,699	5,416	1,951
August ..	8,346	4,252	2,035	10,069	4,958	1,787	9,889	5,430	1,768
September ..	8,254	3,964	1,814	10,416	4,575	1,838	12,009	5,786	1,819
October ..	8,251	4,047	1,508	9,761	4,459	1,653	11,616	5,459	1,785
November ..	7,878	3,605	1,598	8,962	3,975	1,530	10,900	4,829	1,569
December ..	7,322	3,433	1,220	8,954	4,312	1,473	10,713	4,707	1,414
TOTALS ..	87,560	47,128	23,790	108,524	53,419	21,266	130,414	63,706	20,276
YEARLY GRAND TOTAL.		158,532			183,209			214,396	

Average actual labour at work per year, 166,690.

## APPENDIX V.

## Estate 'A'.

## Sickness statistics.

	1926.				1927.			
	TOTAL NUMBER CASES.		TOTAL DAYS LABOUR LOSS DUE TO		TOTAL NUMBER CASES.		TOTAL DAYS LABOUR LOSS DUE TO	
	Malaria.	All diseases.	Malaria.	All diseases.	Malaria.	All diseases.	Malaria.	All diseases.
January ..	77	231	100	575	74	174	133	661
February ..	72	182	120	498	57	129	130	524
March ..	84	171	123	537	62	122	129	476
April ..	61	168	121	577	42	110	106	399
May ..	69	141	145	583	80	160	166	498
June ..	107	231	229	625	114	199	242	552
July ..	85	212	119	674	152	214	395	688
August ..	201	315	425	825	213	318	460	879
September ..	189	288	494	895	202	295	452	778
October ..	184	258	397	732	191	285	441	863
November ..	177	265	362	702	143	241	253	658
December ..	98	193	210	751	113	246	202	830
TOTALS ..	1,404	2,655	2,840	7,974	1,443	2,493	3,109	7,806

## APPENDIX V—concl'd.

	1928.				1929.			
	TOTAL NUMBER CASES.		TOTAL DAYS LABOUR LOSS DUE TO		TOTAL NUMBER CASES.		TOTAL DAYS LABOUR LOSS DUE TO	
	Malaria.	All diseases.	Malaria.	All diseases.	Malaria.	All diseases.	Malaria.	All diseases.
January ..	81	188	174	703	109	183	180	765
February ..	63	148	113	580	72	140	127	546
March ..	52	157	1,119	611	87	168	140	608
April ..	74	146	134	464	144	284	261	810
May ..	75	141	127	540	155	259	337	1,012
June ..	109	203	217	646	146	272	325	1,160
July ..	214	333	505	1,129	204	309	468	1,300
August ..	252	365	650	1,292	203	309	395	1,228
September ..	246	337	582	1,217	275	392	603	1,300
October ..	284	404	488	1,245	313	431	763	1,481
November ..	225	312	417	949	323	393	756	1,269
December ..	135	254	278	822	188	285	316	1,023
TOTALS ..	1,775	2,988	3,804	10,198	2,219	3,425	4,675	12,502

## APPENDIX VI.

## Estate 'A'.

## Sickness statistics—contd.

	1930.				1931.			
	TOTAL NUMBER CASES		TOTAL DAYS LABOUR LOSS DUE TO		TOTAL NUMBER CASES.		TOTAL DAYS LABOUR LOSS DUE TO	
	Malaria.	All diseases.	Malaria	All diseases.	Malaria.	All diseases.	Malaria	All diseases.
January ..	98	189	157	652	69	163	143	455
February ..	82	175	166	587	79	138	141	481
March ..	79	164	138	662	51	113	103	554
April ..	74	171	134	721	115	198	283	1,030
May ..	73	164	112	673	116	243	309	1,385
June ..	168	261	348	925	179	313	396	1,426
July ..	319	409	884	1,532	286	393	756	1,534
August ..	275	373	566	1,247	324	443	886	1,442
September ..	392	509	884	1,524	273	377	711	1,259
October ..	362	471	781	1,520	284	354	753	1,179
November ..	207	312	565	1,236	239	308	595	883
December ..	118	197	278	741	119	217	311	755
TOTALS ..	2,247	3,394	5,013	12,020	2,134	3,260	5,387	12,383

## APPENDIX VII.

## Estate 'B'.

Statistics : Daily average actual labour on books : January 1927 to December 1931.

	1927.	1928.	1929.	1930.	1931.
January ..	519	481	482	522	566
February ..	500	478	489	536	570
March ..	500	494	495	537	586
April ..	514	498	503	552	594
May ..	508	503	516	560	598
June ..	507	506	528	563	604
July ..	505	511	530	574	594
August ..	485	494	522	561	574
September ..	488	498	523	567	571
October ..	488	491	526	566	568
November ..	480	488	525	564	506
December ..	479	481	524	563	506
TOTALS ..	5,969	5,923	6,163	6,705	6,837
Average daily ..	497.41	493.51	513.51	558.75	569.75
Total 300 working days	149,233	148,053	154,053	167,625	170,925

## APPENDIX VII—contd.

*Statistics: Daily average actual labour at work: January 1927 to December 1931.*

	1927.	1928.	1929.	1930.	1931.
January .. ..	329	346	339	367	368
February .. ..	319	334	344	377	358
March .. ..	334	340	349	371	389
April .. ..	353	346	356	387	401
May .. ..	325	363	361	405	400
June .. ..	326	366	368	387	416
July .. ..	369	372	383	407	385
August .. ..	361	366	383	418	390
September .. ..	371	385	388	413	390
October .. ..	363	371	389	417	375
November .. ..	359	373	385	414	331
December .. ..	350	356	377	378	335
<b>TOTALS ..</b>	<b>4,109</b>	<b>4,318</b>	<b>4,422</b>	<b>4,781</b>	<b>4,528</b>
<b>Average daily ..</b>	<b>342.42</b>	<b>359.83</b>	<b>368.50</b>	<b>395.08</b>	<b>377.33</b>
<b>Total 300 working days</b>	<b>102,726</b>	<b>107,949</b>	<b>110,550</b>	<b>118,524</b>	<b>113,199</b>
<b>Percentage labour efficiency.</b>	<b>68.84</b>	<b>72.91</b>	<b>71.76</b>	<b>70.71</b>	<b>66.23</b>

## APPENDIX VIII.

Estate 'B'.

*Sickness statistics.*

	1927.			1928.		
	TOTAL NUMBER CASES		TOTAL DAYS LABOUR LOSS.	TOTAL NUMBER CASES.		TOTAL DAYS LABOUR LOSS.
	Malaria.	All diseases	All diseases.	Malaria.	All diseases.	All diseases.
January ..	65	112	480	56	103	415
February ..	40	81	307	18	96	366
March ..	38	81	346	39	114	458
April ..	62	106	370	47	105	390
May ..	52	97	381	52	152	457
June ..	53	116	417	73	181	485
July ..	57	115	421	139	249	529
August ..	74	115	474	124	231	680
September ..	78	132	521	82	153	410
October ..	95	117	408	107	202	559
November ..	81	122	449	67	132	463
December ..	59	115	419	52	122	377
<b>TOTALS ..</b>	<b>754</b>	<b>1,309</b>	<b>4,993</b>	<b>856</b>	<b>1,840</b>	<b>5,789</b>

## APPENDIX VIII—concl'd.

	1929.			1930.		
	TOTAL NUMBER CASES.		TOTAL DAYS LABOUR LOSS.	TOTAL NUMBER CASES.		TOTAL DAYS LABOUR LOSS.
	Malaria	All diseases.	All diseases	Malaria.	All diseases.	All diseases.
January ..	45	125	418	63	151	509
February ..	36	92	368	38	152	408
March ..	28	75	341	34	136	490
April ..	21	85	311	40	166	571
May ..	141	443	473	109	187	602
June ..	146	511	800	98	277	1,182
July ..	105	323	564	125	345	1,144
August ..	140	421	810	153	364	858
September ..	143	383	815	151	254	1,194
October ..	195	379	907	129	209	1,081
November ..	172	419	676	126	281	690
December ..	135	414	581	197	375	966
TOTALS ..	1,307	3,670	7,064	1,263	2,897	9,695

## APPENDIX IX.

## Estate 'B'.

## Sickness statistics—concl'd.

				TOTAL NUMBER CASES.		TOTAL DAYS LABOUR LOSS.
				Malaria.	All diseases.	All diseases.
1931.						
January ..	..	..	..	181	359	876
February ..	..	..	..	140	387	651
March ..	..	..	..	94	323	567
April ..	..	..	..	96	344	814
May ..	..	..	..	145	423	1,042
June ..	..	..	..	124	284	1,062
July ..	..	..	..	185	472	1,312
August ..	..	..	..	211	371	1,373
September ..	..	..	..	103	306	1,113
October ..	..	..	..	134	356	1,467
November ..	..	..	..	80	343	1,807
December ..	..	..	..	56	195	1,054
TOTALS ..				1,549	4,163	13,138



A MALARIA SURVEY OF KACHUGAON, GOALPARA  
DISTRICT, ASSAM.

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[June 24, 1932.]

INTRODUCTION.

KACHUGAON is a forest settlement, which was established 30 years ago in the Gossaigaon *thana* of the Dhubri subdivision of Goalpara District, 12 miles from the foot of the Bhutan Hills. The settlement covers an area of 205 acres, which contains the bazaar and the residential quarters of the forest officials and petty shop-keepers. It is bounded on the east by an irrigation channel, and on the north, west and south by the Boriali River. The latter is a small stream with a winding course, and water flows in it throughout the year. Several irrigation channels come off from it, and irrigate the surrounding paddy fields. Since the establishment of the settlement a number of villages have sprung up in the immediate neighbourhood, which has led to the progressive extension of paddy cultivation, and the creation of numerous irrigation ditches. There is an abundance of natural undergrowth in and around the settlement, and vast 'Sal' forests extend on every side from within 2 miles of its periphery.

*Area surveyed.*

This includes Kachugaon proper and all the villages situated within a radius of one mile from it (Table I).

*Climate.*

The average mean temperature is approximately 87°F., August being the warmest month (average 100°F.) and December and January the coldest (average 65°F.). The minimum temperature in winter may fall as low as 54°F. The rainy season usually begins in April and continues till October, the average annual precipitation being 170 inches. June is usually the wettest month in the year and December the driest, there being frequently no rainfall at all in the latter month. The atmosphere is generally moist, the monthly mean relative humidity averaging 80 per cent or more from July to November and being as high as 77 per cent in December. Various climatic data are given in Appendix A.

*Population.*

The population of Kachugaon and the neighbouring villages at the census of 1931 is given in Table I.

TABLE I.

Kachugaon and its bazaar	..	..	..	..	215
Bharatnagar	..	..	..	..	292
Dhardhera	..	..	..	..	388
Jalleswari	..	..	..	..	327
Bijohnagar	..	..	..	..	159
Belladong	..	..	..	..	169
Nabinagar	..	..	..	..	56
Raikhambhari	..	..	..	..	226
Rampur	..	..	..	..	210
Malbhog	..	..	..	..	143
Bansbari	..	..	..	..	64
Krishnanagar	..	..	..	..	114

Kachugaon and its bazaar contain heterogeneous population originating from different parts of Assam, Bengal, the United Provinces and Nepal. The surrounding villages are inhabited chiefly by Santals, who have migrated from Santal Parganas District, Bihar. These settlers have been attracted to the neighbourhood by the remission of rent in exchange for manual labour, as contrasted with the conditions in their native districts, where land is scarce.

The residents of Kachugaon proper are Government servants belonging to the Forest Department. The bazaar area is inhabited by petty merchants, whilst the villagers are all cultivators. The villagers are scantily clothed and never use mosquito nets. Their main diet consists of rice and vegetables. The inhabitants of the settlement and bazaar are better fed and better clothed, and almost all of them use mosquito nets of some kind.

*Water supply.*

Water for domestic purposes is obtained from shallow wells. The river water is used for bathing and washing purposes, and also for watering cattle.

*Vital statistics.*

These are not properly maintained, and it was found impossible to obtain figures for the individual villages. The figures given in Table II were furnished by the *mauzadar*,\* and refer to Kachugaon and certain villages which come under the same collecting officer.

TABLE II.

Year.	Total births.	Total deaths.	Deaths from 'fever'.	Infant deaths.
1926 .. ..	225	129	106	32
1927 .. ..	244	178	126	22
1928 .. ..	160	142	84	23
1929 .. ..	274	113	92	19
1930 .. ..	296	182	162	38
1931 . . .	343	210	189	33

The figures representing infant mortality give an average of 111 deaths for every 1,000 births registered, compared with the provincial figure of 174 for the year 1930. As the registration of births and deaths is not compulsory, not much importance can be attached to these figures. Still-births are not recorded.

As regards the age incidence of mortality, the largest number of deaths occur between the ages of one and five years. The great majority of deaths are registered under the heading of 'fever', and, in the writer's opinion, approximately 90 per cent of these are attributable to malaria.

During the period 1924-1931 there have been 21 cases of blackwater fever (Table III).

TABLE III.

Year.	Number of black- water fever cases
1924 .. ..	7
1925 .. ..	1
1926 .. ..	0
1927 .. ..	0
1928 .. ..	1
1929 .. ..	2
1930 .. ..	9
1931 .. ..	1

\* An official in charge of a circle of villages.

## SUMMARY OF PREVIOUS SURVEY (1926).

A short survey of Kachugaon proper was made by the writer in September 1926. The spleen rate was 95·8 per cent (72 observations).

The following species of anopheline mosquitoes were met with:—*A. minimus*, *A. aconitus*, *A. hyrcanus* var. *nigerrimus*, *A. barbirostris*, *A. fuliginosus*, *A. vagus*.

Certain recommendations were made, viz., improvements in drainage, filling up of borrow-pits, provision of a piped water supply, improvements in the conservancy system, electrification of the residential quarters, felling of trees and undergrowth, clearing the banks of the river, provision of a sluice-gate to maintain the height of the river level, and to allow the fish to prey on the mosquito larvæ, and the clearing of all jungle within a radius of half a mile.

## RESULTS OF THE PRESENT INVESTIGATION.

*Examination of children.*

Altogether 528 children between the ages of two and ten years were examined for enlargement of the spleen and for parasites in the peripheral blood. Of these, 450 (85·2 per cent) were found to have splenic enlargement, and 370 (70 per cent) were found to have malaria parasites in the blood. In addition, 26 children below the age of 2 years were examined, and all of these were found to have enlarged spleens, and to have parasites in their blood. The details regarding the spleen and parasite findings among children are given in Appendix B (6), from which it will be seen that both spleen and parasite rates are highest among the youngest children and diminish progressively up to the age of ten years.

The spleen rate among 43 children living in Kachugaon proper was 67·4 per cent, and the parasite rate 32·5 per cent [Appendix B (8)].

The proportions of the various species of parasite found were as follows:—

Malignant tertian	..	..	..	47·4 per cent
Benign tertian	..	..	..	39·7 "
Mixed B.T. and M.T.	..	..	..	11·9 "
Quartan	..	..	..	1·1 "

*Examination of adults.*

Altogether 805 adults of both sexes were examined for enlargement of the spleen, and blood films were taken from 227 of these. The adult spleen rate was 41·7 per cent, and the adult parasite rate 39·6 per cent. The spleen rate amongst adults was much higher among the immigrant population than among the indigenous inhabitants, being 71 per cent in the former case and 29·5 per cent in the latter [Appendix B (10)].

*Anopheline mosquitoes.*

In the course of the survey the following species of anopheline mosquitoes were encountered :—

1. *A. minimus* Theobald.
2. *A. aconitus* Donitz.
3. *A. hyrcanus* var. *nigerrimus* Giles.
4. *A. barbirostris* Van der Wulp.
5. *A. fuliginosus* Giles.
6. *A. vagus* Donitz.
7. *A. subpictus* Grassi.
8. *A. culicifacies* Giles.
9. *A. listoni* Liston.
10. *A. philippinensis* Ludlow.
11. *A. maculatus* Theobald.
12. *A. kochi* Donitz.

Only the first six of these were found in the survey of 1926.

The results of the catches of adult anophelines made during the course of the survey are given in Table IV. It will be noted that *A. vagus*, *A. minimus* and *A. hyrcanus* were by far the commonest species. The identification of each species was confirmed by the Central Malaria Bureau, Kasauli.

TABLE IV.  
*Catches of adult mosquitoes.*

Species.	Number caught.	Percentage of each species.
<i>A. vagus</i> ..	6,511	56·39
<i>A. minimus</i> ..	3,178	27·52
<i>A. hyrcanus</i> ..	1,322	11·45
<i>A. fuliginosus</i> ..	231	2·00
<i>A. philippinensis</i> ..	69	0·59
<i>A. subpictus</i> ..	64	0·57
<i>A. aconitus</i> ..	64	0·57
<i>A. kochi</i> ..	49	0·42
<i>A. barbirostris</i> ..	30	0·26
<i>A. maculatus</i> ..	22	0·19
<i>A. listoni</i> ..	5	0·04
<i>A. culicifacies</i> ..	1	..

The results of dissections of anopheline mosquitoes are given in Table V. All the gland infections and some of the gut infections were confirmed by the Central Malaria Bureau. The first infected mosquito was caught early in August. It is considered that *A. minimus* is the principal, if not the sole, carrier of malaria in the locality.

TABLE V.  
*Results of dissections of anopheline mosquitoes.*

Species.		Number dissected.	Number with oöcysts in mid-gut.	Oöcyst rate.	Number with sporozoites in salivary glands.	Sporozoite rate.
<i>A. minimus</i>	..	1,119	91	8.13	9	0.89
<i>A. vagus</i>	..	451	0	..	0	..
<i>A. aconitus</i>	..	5	0	..	0	..
<i>A. fuliginosus</i>	..	4	0	.	0	..
<i>A. maculatus</i>	..	4	0	..	0	..
<i>A. barbirostris</i>	..	2	0	..	0	..
<i>A. kochi</i>	..	2	0	.	0	..
<i>A. subpictus</i>	..	2	0	.	0	..

*Breeding places of anopheline mosquitoes.*

*A. minimus* requires clear water to breed in, and the larvæ are chiefly found at the grassy edges of hill streams and irrigation channels. They are also found in borrow-pits, ponds and rice fields provided that the water is fairly clear, but are never found in foul water. *A. minimus* does not breed in water which is never exposed to the direct rays of the sun. The larvæ were never found in wells or in pots or in other artificial water receptacles.

The extensive grassy margins of the Boriali River afford ideal permanent breeding grounds for *A. minimus*. Temporary breeding places are created by the April showers, and these persist till the end of October.

No larvæ of *A. culicifacies* or *A. listonii* were found, and those of *A. philippinensis* were only encountered in August. Larvæ of the remaining species were present in various breeding places throughout the investigation.

*Larvicidal fish.*

The following species of larvæ-eating fish were met with during the survey in the streams, irrigation channels and paddy fields :—

1. *Ophiocephalus* sp.
2. *Nandus marmoratus*.
3. *Barbus phutunia*.
4. *Chela* sp.
5. *Trichogaster chuna*.
6. *Haplochilus lineolatus*.
7. *Haplochilus panchax*.

These are present in fair numbers, in the locality, and all of them have been found to prey on larvæ.

*Consumption of quinine and cinchona febrifuge.*

The amount of these drugs expended from the local dispensary during the period 1926–1931 is given in Table VI.

TABLE VI.

Year.			Cinchona febrifuge, lbs.	Quinine, lbs.
1926	..	..	6	1212
1927	..	..	9	16
1928	..	..	3	12
1929	..	..	5	2775
1930	..	..	2	1756
1931	..	..	..	1456

Nearly three-quarters of the above amounts are consumed by the Government staff at Kachugaon, the villagers being less willing to take the drugs.

*Seasonal distribution of malaria.*

The number of cases of malaria treated monthly at Kachugaon dispensary during the period 1926–1931 are given in Appendix B (7). The largest number of malaria cases usually occur in May, i.e., about one month after the onset of the rains. In 1929, when 5 inches of rain fell in January (usually a dry month) the fever peak was in February. The year 1928 differed from all the others in showing the maximum number of cases in November.

The least malarious months are August, September and October, i.e., the period following the torrential rains of June and July, when the water in the streams is laden with silt.

*Possibility of importation of malaria from outside the settlement.*

At the beginning of each winter a floating population of about 500 men with their families comes into the district from highly malarious areas in Bengal, Nepal and the United Provinces, and settles in various parts of the forest. These people visit the local market, which is held weekly on Sundays, and spend Saturday night at Kachugaon. It is probable that the weekly visit of these highly infected persons plays an important part in the dissemination of malaria among the local inhabitants.

*Cost of malaria in Kachugaon.*

It is extremely difficult to estimate the annual cost of malaria in the Kachugaon area. An approximate figure may be arrived at from a study of the records of Kachugaon dispensary. Most of the patients treated here are Government servants, and the average number of cases treated annually for malaria for the years 1923-1930 was 1,744. If we take the average earning capacity of each individual to be Rs. 30 per month (a moderate estimate) and assume that each attack of fever entails the loss of one week's work, we arrive at a figure of Rs. 12,208 as the annual loss in wages. To this must be added the cost of medical aid and drugs, the amount lost by granting sick leave, and the loss sustained by individuals in sending away their families to healthier places. The indirect effects of malaria in forming an important predisposing cause of other diseases must also be taken into account. It is considered that at a moderate estimate the annual cost of malaria in Kachugaon is not less than Rs. 25,000 per annum.

## RECOMMENDATIONS.

*Staff and anti-malarial apparatus.*

It is considered that the appointment of a trained whole-time malaria officer is essential. For anti-larval work a gang of 8 coolies working under 2 malaria supervisors would be required. The annual cost under this head is estimated as follows :—

	Rs.
Anti-malaria Officer at Rs. 150-200 per month	1,800-2,400
2 Supervisors at Rs. 25 per month each ..	.. 600
8 coolies at Rs. 20 per month each ..	.. 1,920
Total Rs.	4,320-4,920

The annual cost of apparatus and material for the application of Paris green and oils is estimated at approximately Rs. 3,200.

The total annual recurring cost of anti-larval measures would thus be about Rs. 8,000.



*Control of breeding places.*

The most important source of breeding of *A. minimus* is the Boriali River. It is suggested that a bund be built across the river below the settlement in order to keep the river full of water all the year round, and to enable the larvæ-eating fish to prey on the mosquito larvæ. The banks of the river should be kept cleared of grass, and destruction of small fish should be prohibited.

Temporary breeding places should be treated with Paris green.

*Mosquito-proofing of residential quarters.*

At present the bungalows of the Divisional Forest Officer and the Extra Assistant Conservator of Forests are fitted with wire gauze, but the work has not been carried out in a proper manner. One defect is that the mosquito-proof doors and windows open inwards instead of outwards.

All residential quarters should be screened with mosquito-proof gauze in such a manner as to prevent all mosquitoes from entering.

*Provision of electric fans in quarters and offices.*

This would be of great service, especially as the mosquito-proofing of the buildings will interfere to some extent with the circulation of air. The breeze from the fans will also tend to keep mosquitoes from biting.

*Mass treatment with plasmoquine.*

In recent years it has been shown that plasmoquine is not only a valuable drug in the treatment of malaria, but that it also renders persons with malarial parasites in their blood non-infective to mosquitoes. It is suggested that mass treatment with this drug be carried out during the winter months.

SUMMARY.

1. Malaria is a serious cause of sickness and mortality in Kachugaon and the surrounding villages.
2. The annual importation of labourers from hyperendemic areas is an important factor in keeping up the high incidence of the disease.
3. *A. minimus*, which breeds freely in the streams and irrigation channels, is the principal, if not the sole, carrier of malaria in the area.
4. It is considered that the incidence of malaria can be very greatly reduced by a systematic campaign directed against the breeding places of *A. minimus*.
5. For this purpose an anti-malarial staff under a whole-time malaria officer is essential. The annual recurring cost of the staff and the necessary appliances is estimated at approximately Rs. 8,000.
6. It is also suggested that mass treatment of the population with plasmoquine be carried out.
7. Other recommendations include mosquito-proofing of residential quarters and offices, and the provision of electric fans.

## APPENDIX A (1).

*Statement showing the monthly rainfall in inches at Kachugaon in the district of Goalpara, Assam, for the years 1926-1931.*

Month.	YEAR.					
	1926.	1927.	1928.	1929	1930.	1931.
January ..	..	0·70	0·05	4·18	..	..
February ..	0·25	0·62	0·11	..	0·92	0·50
March ..	4·98	3·42	0·63	6·69	4·68	1·39
April ..	3·54	2·41	8·0	13·90	9·57	15·95
May ..	12·71	17·25	22·86	22·82	15·26	21·28
June ..	47·49	60·23	26·36	62·71	44·71	53·50
July ..	46·38	36·52	35·42	28·27	5·72	48·80
August ..	15·47	15·76	36·70	15·65	28·86	14·59
September ..	9·46	44·39	31·46	13·63	11·85*	32·48
October ..	7·23	8·55	17·91	20·48	8·63	0·013
November ..	Nil	1·03	0·23	..	2·10	..
December ..	..	..	..	..	..	0·34

## APPENDIX A (2).

*Data regarding temperature and atmospheric humidity at Kachugaon and at Dhubri meteorological observatory recorded during the year 1931.*

Month.	Mean temperature at Kachugaon, °F.	TEMPERATURE AT DHUBRI.		Mean relative atmospheric humidity at Dhubri.* Per cent.
		Max. °F.	Min. °F.	
January ..	75	..	..	..
February ..	78	..	..	..
March ..	85	..	..	..
April ..	90	..	..	..
May ..	97	..	..	..
June ..	91	..	..	..
July ..	95	86	78	85
August ..	99	90	83	86
September ..	93	84	76	85
October ..	94	83	74	82
November ..	77	77	65	80
December ..	68	74	57	77

\* Average of 9 a.m. and 5 p.m. readings.

## APPENDIX A (3).

*Statement showing total number of cases treated in the Kachugaon dispensary, in the district of Goalpara, Assam.*

Month.	YEAR.					
	1926.	1927.	1928.	1929.	1930.	1931.
January ..	702	601	423	435	456	378
February ..	663	543	358	410	427	382
March ..	827	622	463	560	544	434
April ..	464	618	410	570	592	479
May ..	709	634	422	590	748	589
June ..	710	574	499	587	586	604
July ..	668	594	578	548	501	597
August ..	702	488	554	561	395	584
September ..	643	487	604	496	307	656
October ..	489	390	632	399	526	506
November ..	462	407	1,001	504	582	608
December ..	513	458	887	489	372	447

## APPENDIX B (1).

*Statement showing the births in the Kachugaon Forest settlement in the district of Goalpara, Assam.*

Month.	YEAR.					
	1926.	1927.	1928.	1929.	1930.	1931.
January ..	8	16	10	8	36	42
February ..	28	13	1	7	19	38
March ..	10	20	9	13	19	30
April ..	11	16	8	13	16	27
May ..	9	20	10	7	30	28
June ..	4	12	12	8	28	16
July ..	14	24	13	22	28	15
August ..	10	60	29	21	20	24
September ..	30	21	17	40	24	35
October ..	45	13	19	51	20	30
November ..	34	11	14	45	31	45
December ..	22	18	18	39	25	13

## APPENDIX B (2).

*Statement showing total deaths in the Kachugaon Forest settlement in the district of Goalpara, Assam.*

Month.	YEAR.					
	1926.	1927.	1928.	1929	1930.	1931.
January ..	4	17	4	3	13	27
February ..	11	9	3	4	10	14
March ..	8	6	4	4	15	16
April ..	11	6	19	3	14	10
May ..	10	18	22	4	25	32
June ..	6	32	17	6	14	19
July ..	21	12	15	15	11	15
August .	9	34	25	5	9	22
September .	4	13	3	17	16	24
October ..	19	11	15	15	22	9
November .	18	14	5	16	13	9
December .	8	6	10	21	10	13

## APPENDIX B (3).

*Statement showing total deaths from malaria in the Kachugaon Forest settlement in the district of Goalpara, Assam.*

Month.	YEAR.					
	1926.	1927.	1928.	1929.	1930	1931.
January ..	4	13	4	3	12	26
February ..	10	8	3	4	9	10
March ..	8	3	4	2	12	16
April ..	2	6	6	3	14	9
May ..	5	18	9	4	25	28
June ..	6	24	8	5	14	14
July ..	17	6	8	12	10	14
August ..	8	14	18	4	9	19
September ..	4	11	..	10	16	24
October ..	17	11	12	11	18	7
November ..	18	7	2	13	13	9
December ..	7	5	10	21	10	13

APPENDIX B (4).

*Statement showing the mortality among infants in the Kachugaon Forest settlement in the district of Goalpara, Assam.*

Month.	YEAR.					
	1926.	1927.	1928.	1929.	1930.	1931.
January ..	1	3	..	1	2	8
February ..	1	1	..	1	4	5
March ..	..	1	1	..	..	1
April ..	1	..	..	1	2	1
May ..	1	2	3	2	11	8
June ..	2	6	3	..	3	1
July ..	7	1	5	1	4	..
August ..	5	4	3	2	4	1
September ..	3	2	..	3	1	1
October ..	5	2	3	1	3	0
November ..	5	..	1	3	6	4
December ..	1	..	5	4	2	3

APPENDIX B (5).

*Statement showing age incidence of mortality at Kachugaon Forest settlement of the district of Goalpara, Assam.*

Age up to	YEAR.					
	1926.	1927.	1928.	1929.	1930.	1931.
5	19	53	29	28	25	45
10	7	24	9	15	14	26
15	5	9	3	3	7	7
20	7	10	7	6	10	10
30	15	10	12	18	25	22
40	14	15	20	9	20	33
50	6	7	11	5	3	8
60	13	7	13	1	12	14
Over 60	5	10	11	9	18	18

## APPENDIX B (6).

*Statement showing spleen and parasite rates by age groups among the children examined at Kachugaon Forest settlement in the district of Goalpara, Assam.*

Age.	SPLEEN EXAMINATION.			BLOOD EXAMINATION.		
	Number examined for enlarged spleen.	Number with enlarged spleen.	Percentage found to have enlarged spleen.	Number examined for parasites	Number found with parasites.	Percentage found with parasites.
2-4	145	134	92.41	145	115	79.31
5-6	159	142	89.30	159	116	72.95
7-8	124	104	83.87	124	79	63.70
9-10	100	70	70.00	100	60	60.00
TOTAL.	528	450	85.23	528	370	70.07

## APPENDIX B (7).

*Statement showing the total number of malaria cases treated in the Kachugaon Forest dispensary in the district of Goalpara, Assam.*

Month.	YEAR.					
	1926.	1927.	1928.	1929.	1930.	1931.
January ..	201	223	167	118	104	120
February .	143	148	94	212	72	91
March ..	267	113	144	112	102	111
April ..	155	188	135	150	125	149
May ..	185	177	144	133	171	197
June ..	160	134	131	135	111	130
July ..	129	113	156	100	85	157
August ..	158	122	113	84	81	130
September ..	138	123	76	99	61	164
October ..	110	101	180	65	57	175
November ..	160	120	323	93	90	148
December ..	156	141	237	115	82	142
TOTALS .	1,962	1,703	1,900	1,416	1,141	1,714

## APPENDIX B (8).

*Spleen rates by age groups among children at Kachugaon proper.*

Age group (years).	Number examined.	Number found with enlarged spleen.	Percentage found with enlarged spleen.	REMARKS.
2-4 .. ..	10	6	60	Imported.
	3	3	100	Indigenous.
5-6 .. ..	4	1	25	Imported.
	7	6	85·7	Indigenous.
7-8 .. ..	8	5	62·5	Imported.
	4	2	50	Indigenous.
9-10 .. ..	4	3	75	Imported.
	3	3	100	Indigenous.
Total imported ..	26	15	57·7	
Total indigenous ..	17	14	81·2	
TOTAL ..	43	29	67·4	

*Note*—Of the above 43 children, 14, or 32·5 per cent, were found to have malaria parasites in their blood.

## APPENDIX B (9).

*Spleen rates by age groups among adults at Kachugaon proper.*

Age group (years).	Number examined.	Number found with enlarged spleen.	Percentage found with enlarged spleen.	REMARKS.
11-15 .. ..	1	1	100	Imported.
16-20 .. ..	9	6	66·6	Imported.
21-30 .. ..	15	9	60	Imported.
31-40 .. ..	13	4	30·8	Imported.
41-50 .. ..	3	0	..	Imported.
	1	0	..	Indigenous.
Total imported ..	41	20	47·6	
Total indigenous ..	1	0	..	
TOTAL ..	42	20	47·6	

*Note*.—Of the above 42 adults, 10, or 23·8 per cent, were found to have malaria parasites in their blood.

## APPENDIX B (10).

*Spleen rates by age groups among adults in Kachugaon Forest settlement.*

Age group (years).	Number examined.	Number found with enlarged spleen.	Percentage found with enlarged spleen.	REMARKS.
11-15 .. ..	9	8	88·8	Imported.
	23	8	34·8	Indigenous.
16-20 .. ..	19	13	68·4	Imported.
	52	25	48·1	Indigenous.
21-30 .. \ ..	109	81	74·3	Imported.
	209	69	33·0	Indigenous.
31-40 .. ..	88	58	65·9	Imported.
	161	35	21·7	Indigenous.
41-50 .. ..	11	7	63·6	Imported.
	100	25	25·0	Indigenous.
51 and over ..	3	2	66·6	Imported.
	21	5	23·8	Indigenous.
Total imported ..	239	169	70·7	
Total indigenous ..	566	167	29·5	
TOTAL ..	805	336	41·7	

*Note.*—The blood of 227 of the above adults was examined, and malaria parasites were found in 90, or 39·6 per cent.



## PRINCIPLES TO BE OBSERVED IN THE PREPARATION OF CANAL PROJECTS AND IN THEIR EXECUTION.\*

[July 14, 1932]

THESE principles represent a standard to be aimed at, but not necessarily to be rigidly followed where other important considerations are involved. Item No. 7 requires to be particularly brought to the attention of Colonization Officers.

2. All irrigation channels, including main canals, branches and distributaries, shall be aligned, as far as possible, along watersheds.

3. When a canal project is under preparation the importance for providing for drainage shall be borne in mind, and provision should be made to enable a system of drains and outfalls to be undertaken along alignments laid down in the project.

4. All main canals and branches should be so designed that the full supply level of the water shall be as little as possible above that of the adjoining natural surface.

5. Where canals of any size cross a natural drainage or depression, adequate arrangements should be made to pass storm water under or into the canal. When heading up of the water is necessary to pass the water into or under the canal, due regard must be paid to the character of the depression in order that too large a pond is not formed. Similarly, where bridge ramps tend to pond up water, a culvert of suitable size under the ramps must be provided.

6. No weirs shall be constructed on rivers where they are likely to be injurious to large towns or cities in the vicinity. If the construction of a weir in such a situation be unavoidable, suitable provision for anti-water-logging measures shall be provided in the project.

7. Where borrow-pits are necessary for the completion of embankments, they shall, so far as possible, be dug within the canal bed.

8. Main canals and branches should not, if possible, be taken within 2 miles of an existing town nor should a new town be built within 3 miles of a main canal or branch, nor should town extensions be allowed to extend within 2 miles thereof.

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\*From Proceedings of the Water-logging Board, Punjab, December 6, 1930, and reproduced by kind permission of the Board.

9. Where canals must pass through land which is likely to become water-logged by seepage from these canals, adequate provision for drainage shall be made at the time of the construction of the canal. .

10. Canal irrigation shall not be permitted in areas which are likely to become water-logged in the future.

11. All major canal projects shall be placed before the Water-logging Board for scrutiny, and the opinion of the Board shall be placed on record in the project report.

12. A soil survey based on the chemical analysis of the soil with a view to the determination as to whether the rise of the water table would be accompanied by the spread of alkali should form part of the project.

## A FURTHER NOTE ON MALARIA IN PATIALA STATE.

BY

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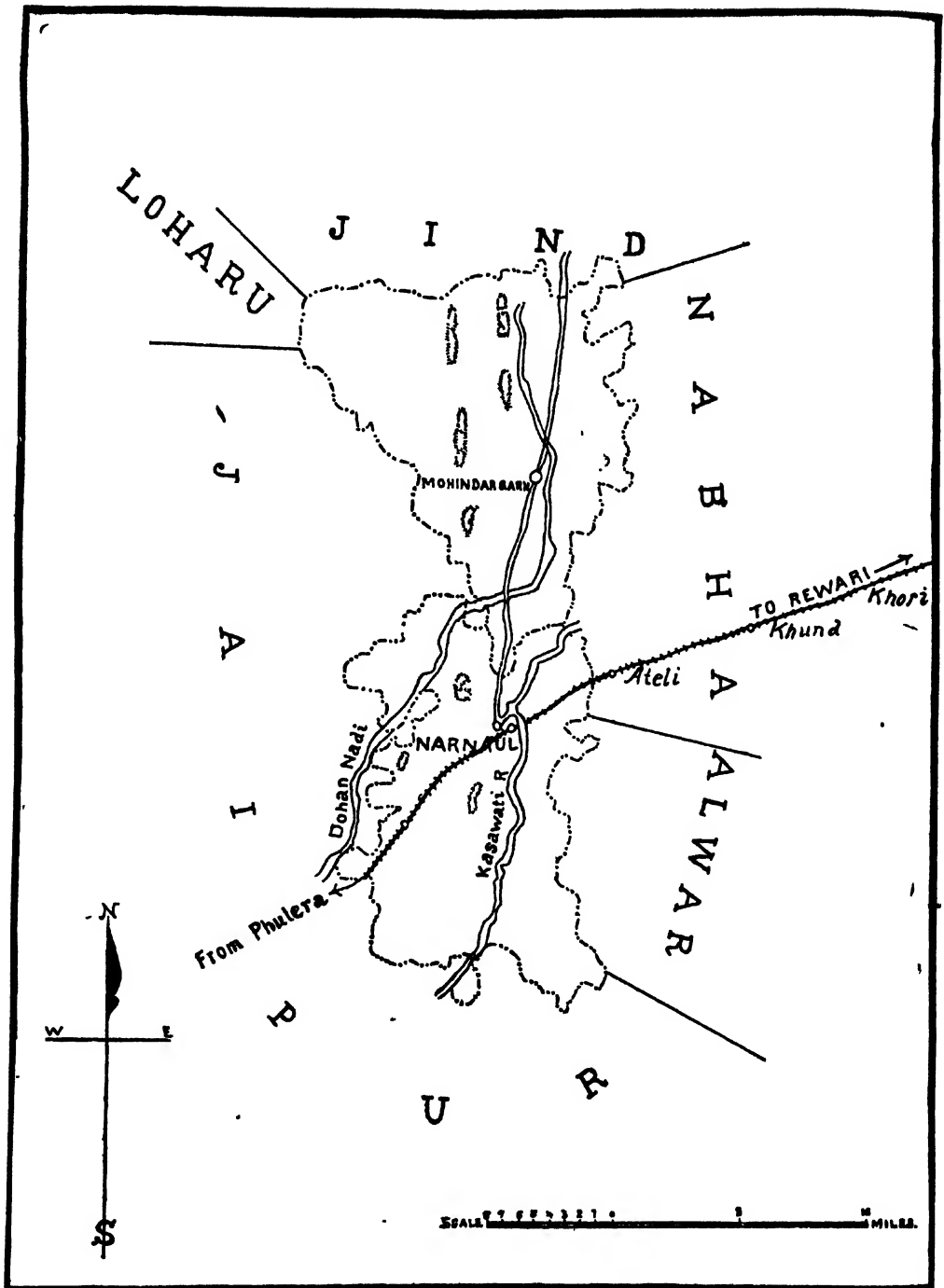
[August 25, 1932.]

A MALARIA survey of Patiala City and of certain other localities in the State was carried out in the year 1931 (Covell, 1932). The observations which form the subject of the present note were made during a subsequent visit to the State in August 1932.

### 1. *Malaria in Narnaul ilaqa (Mohindargarh nizamat).*

This tract of country is entirely separate from the rest of Patiala State, being situated 180 miles south of Patiala City, and 70 miles south-west of Delhi. It was formerly part of the Nawab of Jhajjar's State, but was presented to Maharajah Narindar Singh of Patiala as a reward for his services in the Mutiny. It has an area of 691 sq. miles, measuring 45 miles from north to south and 22 miles from east to west. On the north it is bounded by a piece of territory belonging to Jind State, on the west and south by Jaipur State, and on the east by the State of Alwar and an out-lying block of Nabha State (*see* Map). Geographically it forms part of the Rajputana Desert, and the country is quite unlike most parts of the Punjab, being undulating and sandy, with numerous rocky hills which form part of the Aravalli system. The tract is partially watered by three streams: the Dohan, which rises in the Jaipur hills, traverses the whole length of the *nizamat* and passes into Jind territory to the north; the Krishnavarti, which also rises in Jaipur and flows past Narnaul into Nabha territory to the east; and the Gohli. It is divided into two *tahsils*, Mohindargarh or Kanaud, and Narnaul.

NARNAUL, the headquarters of Narnaul *tahsil*, is a town with 23,000 inhabitants situated 37 miles south-west of Rewari. It is built on high ground, and the houses, some of which have two storeys, are almost all made of stone. Its streets are steep and narrow but paved with stone, and the climate is healthy, being hot and dry. The annual rainfall is about 16 inches.



Map of the *ilaga* of Narnaul (Mehindargarh nizamet).

There are a number of tanks in and around the town, but apart from these there are very few water collections. There is a large river bed (the Chhalak nadi) running through the centre of the town, but it only contains water for very short periods, and was completely dry at the time of my visit, in spite of recent rain. The subsoil water level was between 50 and 60 feet. The chief crops in this area are *jowar* and *bajra*, and the only form of irrigation is from wells.

The splenic index, obtained from the examination of 390 children (chiefly school children), was 2 per cent (Table I). The vast majority of the anophelines caught were *A. subpictus*, but *A. culicifacies*, *A. fuliginosus*, *A. stephensi* and *A. pallidus* were also present in scanty numbers (Table II).

A perusal of the number of cases diagnosed annually as malaria during the last 30 years showed that the town was severely affected at the time of the great Punjab epidemic of 1908 (Table III). It is evident that endemic malaria is normally low, but that the town is subject to periodical outbreaks of the disease in epidemic form.

MOHINDARGARH or KANAUD, the headquarters of the *tahsil* of the same name, is situated 16 miles to the north of Narnaul, and has a population of 9,000. The subsoil water level at the time of my visit was 60 feet, and the general conditions appeared very similar to those at Narnaul. The soil is, if anything, more sandy, and the chief crops are the same. The splenic index among 403 children (chiefly from schools) was 3 per cent (Table I). *A. subpictus* was by far the most prevalent anopheline, *A. culicifacies* and *A. stephensi* being also present in scanty numbers. Except for the village ponds no collections of water were seen. As at Narnaul, the dispensary figures showed that though the incidence of malaria is normally low, the town is subject to periodical outbreaks of epidemic malaria (Table III).

## 2. Malaria in the Ghaggar tract.

Two villages, Badshahpur and Sutariana (or Kharamgarh), were visited in this area.

The present course of the Ghaggar River runs along the border of the State where it joins Karnal district. The river here runs in a narrow channel 40 to 60 feet deep, which was almost full at the time of my visit. The former bed of the river, known as the Old Ghaggar, pursues a tortuous course more or less parallel with this, and is connected with the present river, so that it and its tributaries now form backwaters, which contain slowly-flowing or stagnant water during the rains. As the river falls, pools are formed in the Old Ghaggar bed.

The bed of the Ghaggar in this district is so deep that the water does not flood the countryside except on very exceptional occasions, about once in 10 years. When such floods occur they are welcomed by the cultivators, as it is then possible to raise a heavy crop of rape from the silt-covered land when the floods have subsided.

The chief crops are rice and cotton, and these are irrigated not from the Ghaggar, but from the Kharamgarh branch of the Sirhind system of canals. The whole tract has a reputation for being very malarious, and the *zamindars* say that the cultivation of the land is seriously affected on this account.

BADSHAHPUR is a small village with about 100 inhabitants, situated about 300 yards from the Old Ghaggar bed. It is built on an elevated site raised some 25 feet above the surrounding country, so that while the subsoil water level at the time of my visit was 27 feet, the water in the village well was more than 60 feet below the surface of the ground. The soil is chiefly clay. The present bed of the Ghaggar is about 1,000 yards distant, and an irrigation channel from the Kharamgarh *rajbaha* runs close by the village, being carried over the Old Ghaggar bed by an aqueduct.

Out of 60 children from Badshahpur and from the neighbouring village of Sona, 38 had enlarged spleens, giving a splenic index of 63 per cent. This figure is in reality too low, as several children who had only recently come to the village and who showed no enlargement of the spleen were included. The Director of Medical Services, Patiala State, found 18 children with enlarged spleens out of 20 examined in September 1931. The general condition of the children was poor, and malaria is evidently very severe.

Adult specimens of both *A. subpictus* and *A. culicifacies* were collected in large numbers in this village, whilst *A. stephensi*, *A. fuliginosus*, *A. pallidus* and *A. pulcherrimus* were also present in scanty numbers.

SUTARIANA, or KHARAMGARH, is a village with a population of 2,000, situated 8 miles south of Badshahpur. The distance from the Old Ghaggar bed is one mile, and from the present river 4 miles. Wet cultivation extends almost to the edge of the village, and there are several large ponds. As at Badshahpur, irrigation is from the Kharamgarh branch of the Sirhind canal system, the chief crops being rice and cotton. The soil is of clay, and the subsoil water level at the time of my visit was 27 feet. Adult specimens of *A. subpictus* and *A. culicifacies* were numerous, and specimens of *A. fuliginosus*, *A. stephensi*, *A. pallidus* and *A. pulcherrimus* were also caught. The splenic index (100 observations) was 26 per cent.

The conditions in this tract of country strongly resemble those in certain parts of Sind, where former beds of the Indus (locally known as 'dhoros') are present. Villages situated along the course of these channels are invariably intensely malarious, the amount of malaria becoming less as the distance from the channels is increased. Badshahpur, although it is situated on high ground and has no wet cultivation in its immediate vicinity, presents a typical example of hyperendemic malaria. Sutariana is much less malarious, although there is wet cultivation close to the village. There is no doubt that the greater intensity of malaria at Badshahpur is due to its close proximity to the old bed of the Ghaggar River.

Anti-larval measures in the villages of the Ghaggar tract are out of the question, and the only measure available for the mitigation of malaria in this

part of the State is the provision of increased facilities for the treatment of the disease. This could best be secured by the provision of one or more travelling dispensaries. Failing this, arrangements should be made for the supply of an adequate amount of quinine or cinchona febrifuge to each village.

### 3. Malaria at Rajpura.

RAJPURA, the headquarters of the Pinjaur *nizamat* and Rajpura *tahsil*, is situated 16 miles north-east of Patiala City, and has a population of 4,000. It has a station on the North-Western Railway, and is the junction for the Rajpura-Bhatinda branch. The principal crops are rice, maize, sugar-cane, wheat and gram. There is no perennial irrigation, but the rice crop is raised by water conveyed by inundation channels from the Ghaggar River, which runs 12 miles from the town. One of these channels comes into the town itself close to the dispensary. The winter crops are watered by irrigation from wells. The subsoil water level at the time of my visit was 48 feet.

There were numerous water collections in and around the village, chiefly in borrow-pits along the sides of the roads and railway line. The drainage of the town is bad, and the sullage water from the houses is led into sumps ('choubachas').

The splenic index among 249 children (chiefly from schools) was 14 per cent. Adult specimens of *A. subpictus* and *A. culicifacies* were numerous, and specimens of *A. stephensi*, *A. fuliginosus*, *A. pallidus* and *A. pulcherrimus* were also caught. Culicine mosquitoes were present in enormous numbers, and the people complained of the 'mosquito nuisance' rather than of malaria.

The following measures are recommended :—

(1) Borrow-pits should be filled up as much as possible. There are numerous mounds of earth and broken brick in and around the town which can be used for this purpose, and town rubbish may also be used, being covered with a layer of earth rammed down. The smaller pits should first be filled, and the water in the larger ones treated with crude oil or waste motor oil until they also can be filled. A knapsack oil sprayer should be provided for this purpose. Arrangements could probably be made for the Sub-Assistant Surgeon in charge of the anti-malaria work in Patiala City to initiate the work, or the Assistant Surgeon in charge of the dispensary at Rajpura could be sent to Patiala for a few days to learn the necessary practical details.

(2) The sumps into which sullage water from the houses is drained should be treated regularly with oil once a week, for these are undoubtedly a major source of the breeding of culicine mosquitoes.

(3) Wells used for drinking water may be treated with petrol. The amount to be used is 24 ozs. per 80 square feet of water surface. This should be applied once a week at night, so that by the next morning any taste of petrol in the water will have disappeared.

**4. Anti-malarial measures in Patiala City.**

As the result of the survey carried out in 1931 certain recommendations were made as regards anti-malarial measures. The chief of these was the abolition of wet cultivation within the city boundaries, and this was urged not only as an anti-malarial measure, but also from the view of general sanitation. This measure has resulted in a great improvement in health in other localities, notably in the case of Saharanpur City, which was formerly very malarious.

In the case of Patiala City it appears that there are certain difficulties in enforcing such a measure. In the course of an informal discussion with some of the members of the Municipal Committee, it was suggested that the desired result might be attained by a substantial increase in the water rate charged for irrigating wet crops within the city limits, the charge for water used in cultivating fruit trees being kept at the former level. It is probable that wet cultivation in this area would cease if this were done, without any loss in revenue to the State, and it is to be hoped that regulations will be passed to this effect in the near future.

As regards other measures, the chief breeding places of anopheline mosquitoes are being treated with Paris green or oil, but the permanent measures recommended are being delayed owing to the financial stringency. It is realized that this is inevitable, but I would press that one urgent work should be carried out without delay. This is the rendering 'pukka' of the channel which supplies water to the Rajinder tank. The channel runs immediately behind the hospital, and is a dangerous source of anopheline breeding. It is comparatively short, and the work should not present great difficulty, as it was originally brick-lined.

*Summary.*

1. An account is given of certain observations carried out in Patiala State in August 1932.

2. Endemic malaria in the *ilaga* of Narnaul, which is situated 60 miles to the south of the main block of Patiala territory, is very slight, but the tract is subject to periodical outbreaks of epidemic malaria.

3. Malaria in the Ghaggar tract is hyperendemic. The intensity of the disease in this area is held to be due to the presence of channels representing the former bed of the Ghaggar River. Increased facilities for the treatment of malaria, including the provision of one or more travelling dispensaries, are recommended for this area.

4. Recommendations are made with regard to anti-malarial measures in Rajpura and in Patiala City. In the case of the latter it is urged that an attempt be made to abolish wet cultivation within the city limits by a revision of the water rates charged for irrigation of wet crops.

## - REFERENCE.

- COVELL, G. (1932) .. .. Malaria in Patiala State. *Rec. Mal. Surv. Ind.*, **3**, 1, pp. 83-101.



TABLE I.

*Results of spleen examinations, August 1932.*

Locality.	Number of children examined.	Number with enlarged spleen.	Splenic index.	Size of average enlarged spleen in cm. (A-U measurement).
Narnaul .. ..	390	8	2	10.1
Mohindargarh .. ..	403	13	3	10.6
* Badshahpur and Sona ..	60	38	63	....
* Sutariana .. ..	100	26	26	....
Rajpura .. ..	249	36	14	10.0

\* *Note.*—The sizes of spleens in these localities were measured in finger-breadths only (costal margin projection). The results were as follows:—

	P	1F	2F	3F	4F	U	Total.
Badshahpur and Sona ..	10	8	14	3	1	2	38
Sutariana .. ..	9	10	5	2	..	..	26

TABLE II.

*Result of catches of adult anophelines, August 13-22, 1932.*

Locality.	<i>culicifacies.</i>	<i>subpictus.</i>	<i>fuliginosus.</i>	<i>stephensi.</i>	<i>pallidus.</i>	<i>pulcherrimus</i>
Narnaul ..	5	Very numerous.	2	12	1	0
Mohindargarh	2	Very numerous.	0	2	0	0
Badshahpur ..	Very numerous.	Very numerous.	6	5	2	2
Sutariana ..	Very numerous.	Very numerous.	13	3	3	1
Rajpura ..	40	Very numerous.	13	11	4	2
Patiala City— Cattle sheds near canal.	48	Very numerous.	6	0	3	0
Baradari stables.	38	30	4	0	1	0
Hospital wards.	13	16	2	0	0	0

*Notes.*—The above is the result of about 1½ hours search in each locality cited.

TABLE III.

*Number of cases treated annually for malaria at the dispensaries at Narnaul and Mohindargarh, 1901-1931.*

Year.	MALARIA CASES TREATED.		Year.	MALARIA CASES TREATED.	
	Narnaul.	Mohindargarh.		Narnaul.	Mohindargarh.
1901 ..	...	538	1916 ..	1,660	721
1902 ..	....	126	1917 ..	2,532	....
1903	989	921	1918 ..	954	....
1904 .	908	....	1919 ..	....	....
1905 .	434	....	1920 ..	1,989	....
1906	1,433	1,039	1921 ..	1,216	1,065
1907 ..	1,168	721	1922 ..	1,338	724
1908 ..	6,337	2,761	1923 ..	1,640	412
1909 ..	4,028	1,329	1924 ..	2,253	530
1910 ..	1,379	564	1925 ..	2,844	698
1911 ..	965	744	1926 ..	3,427	1,143
1912 ..	860	600	1927 ..	2,055	532
1913 ..	973	623	1928 ..	1,486	846
1914 .	1,272	366	1929 ..	1,922	1,012
1915 ..	838	299	1930 ..	2,863	1,039
			1931 ..	3,915	1,112

# THE STUDY OF A REGIONAL EPIDEMIC OF MALARIA IN NORTHERN SIND.

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## INTRODUCTION

THE epidemic of malaria which forms the subject of this paper occurred in the autumn of the year 1929. Its chief centre was located in the Upper Sind Frontier and Sukkur Districts, where epidemic figures of over 5 were

recorded in 9 talukas, the highest figure (15.2) occurring in Jacobabad. The distribution of the epidemic is shown in Map I, which gives the epidemic figures recorded in the various talukas throughout Sind. In the annual report of the Director of Public Health, Bombay, for 1929, it was estimated that there were 45,600 deaths from malaria in Sind in that year, an excess of 20,500 as compared with the figures for 1928. In the year following the epidemic there was a decrease in the birth rate throughout Sind, this being most marked in the districts of Sukkur, Larkana and Nawabshah.

The year 1929 was in several respects an abnormal one in Sind. An epidemic of cholera in July and August occasioned 3,707 deaths in Larkana District, 668 in Sukkur District and 186 in the Upper Sind Frontier District. There was abnormally heavy rainfall in July and August throughout the province. In normal years the monsoon rainfall in Northern Sind is almost negligible in amount, it being a common occurrence for the total precipitation to be less than 2 inches; but in 1929 amounts varying from 5 to 22 inches were recorded at the various taluka headquarters in Sukkur and the Upper Sind Frontier Districts. Widespread floods, caused partly by excessive rainfall and partly by overflows and breaches in canals due to the high level of the river Indus, resulted in great damage to crops and property. The volume of water in the Indus was further increased by the bursting of the Shyok glacial dam in Kashmir at the end of August. In the Begari Canals Division in Northern Sind, 877 square miles of country were flooded, and 732 square miles were flooded in the Nasrat Canals Division, which is situated further to the south on the left side of the Indus. Extensive floods were also caused in the Shikarpur Canals Division and in the Western Nara Division. Large numbers of people were rendered homeless, necessitating the establishment of refugee camps at Hyderabad, Dadu, Sukkur and Rohri.

#### PREVIOUS EPIDEMICS OF MALARIA IN NORTHERN SIND.

The last great malaria epidemic in Northern Sind occurred in 1917, and previous to this epidemics were recorded in 1906 and 1897. These were fully discussed by Young and Majid (1930). These authors showed that malaria in epidemic form was particularly liable to occur in this region in years of unusual rainfall, in combination with the incidence of high flood levels in the river Indus. They drew attention to the fact that such epidemics appeared to occur at intervals of approximately 10 years, and predicted the early occurrence of another fulminant outbreak.\*

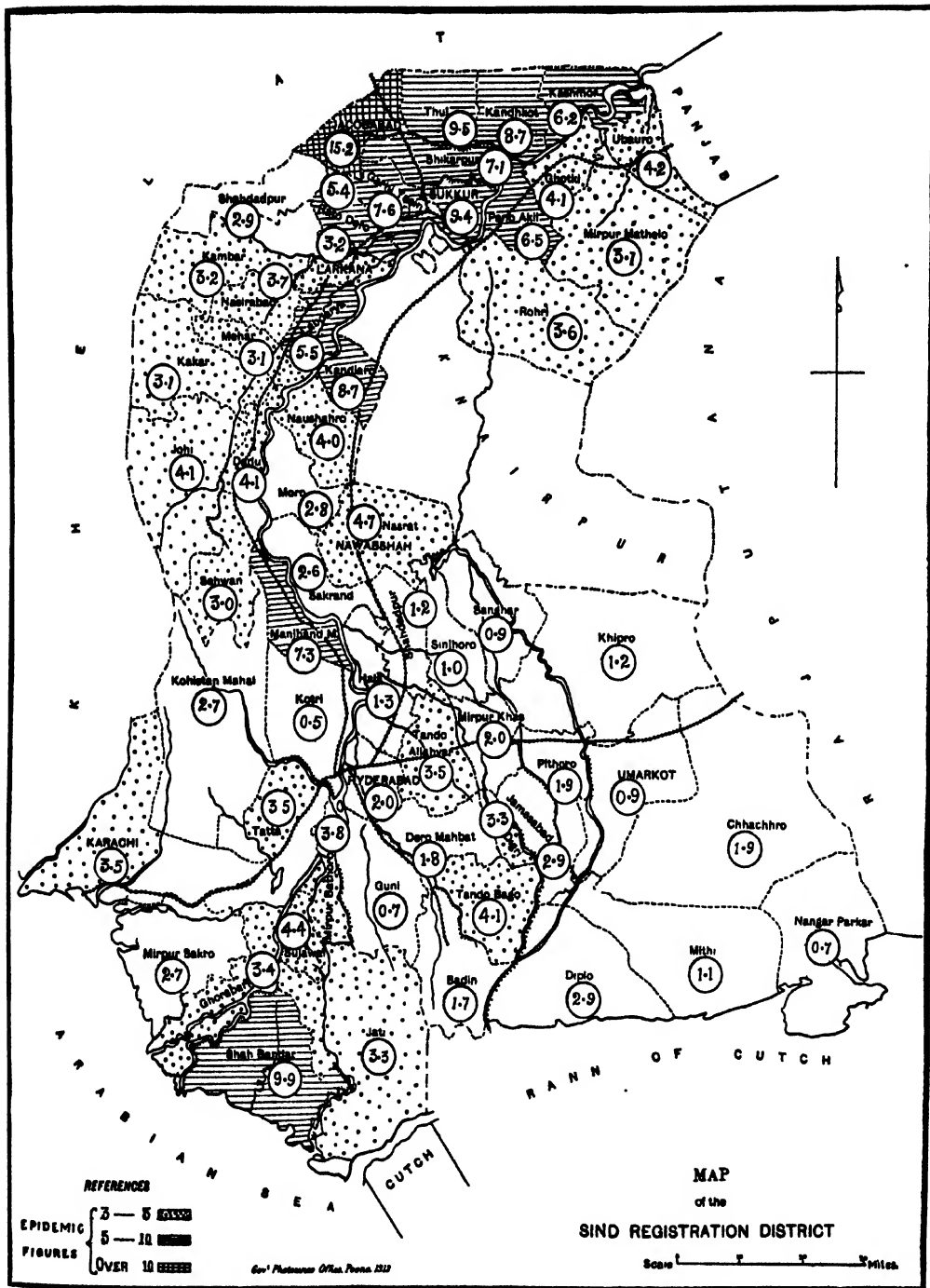
#### THE MECHANISM OF EPIDEMIC MALARIA.

The type of epidemic malaria dealt with in this paper, the distinguishing character of which is its effect on mortality, was first described by Christophers (1911) under the term 'autumnal epidemic (fulminant) malaria'. Gill (1928), who subsequently studied this form of malaria over a period of many years,

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\*The paper referred to was actually written in 1928.

### Taluka Epidemic Figures, 1929



prefers the name 'regional epidemic' in contrast with localized epidemics, due to such causes as 'tropical aggregation of labour', etc. The observations of both these authors were made in the Punjab.

Christophers showed that the essential precipitating cause of epidemic malaria in the Punjab is excessive rainfall, which acts by producing flooding either directly by means of local rain, or indirectly by causing the overflow of rivers. He showed also that adverse economic conditions provide a powerful predisposing cause of fulminant epidemics. In his study of the great epidemic of 1908, he noted that the rise of mortality characteristic of this type of malaria occurred simultaneously in places hundreds of miles apart, and that the focal distribution of the epidemic indicated that it did not depend merely on local causes, but that there must be some general determining influence over and above these. As regards the rainfall, he found that the only relation which seemed to hold between its character and the epidemic conditions was the heaviness of the falls. Whilst admitting that the exact mechanism of epidemic causation was still unknown, Christophers stated that the epidemic condition appears to be due to an excessive seasonal increase of the normal parasite rate, fluctuations in which occur even in healthy years. He urged the importance of the quantitative study of malarial infections, and showed by his experiments with avian malaria in sparrows that severity of infection is dependent not so much on the number of infected mosquitoes as on the number of sporozoites injected at each bite; and that this in turn depends upon the number of gametocytes present in the blood on which the insect has fed.

Gill (1921; 1928), as the result of his study of epidemic malaria in the Punjab, and of his laboratory observations, lays great stress on the effect of meteorological conditions, and especially of atmospheric humidity, in the production of malaria epidemics. He notes that the most striking feature of the atmospheric conditions is the relatively high degree of humidity prevailing during the pre-epidemic period.

Gill holds that an epidemic of malaria is essentially the outcome of a loss of equilibrium between infection and immunity; that is to say, that an epidemic will occur when the conditions become especially favourable for the transmission of infection at a time when the communal immunity is low. The maintenance of high atmospheric humidity in association with high temperature causes an increase in the numerical prevalence, longevity and metabolic activity of the insect carrier, and rapid completion of the sexual cycle of the malaria parasite in the mosquito host. Gill has also suggested that the sudden onset of these climatic conditions may possibly exercise an indirect influence on the malaria parasite in the human host, so that an increased number of parasites become available for transmission.

Macdonald and Majid (1931), in their study of the epidemiology of malaria in the Karnal District (Punjab), showed that marked signs of immunity were present among the older children even after several years during which conditions were unfavourable for the transmission of malaria. Whilst agreeing

in principle with Gill, they are of opinion that the lowered communal immunity is chiefly due to the presence of a large proportion of young children who have never been exposed to infection, and are consequently non-immune. Macdonald and Majid also drew attention to the paucity of gametocyte carriers observed among the population before and at the beginning of the 'malaria season'. They pointed out that at least a month must normally elapse between the date when an anopheline ingests an infective feed and the date of the appearance of gametocytes in the blood of the person subsequently infected by that anopheline; and that therefore a considerable time must elapse before a large population of gametocyte carriers can be created by a process of geometrical progression. They concluded that if the period favourable to transmission is sufficiently prolonged in an area containing a non-immune infant population, a fulminant epidemic will be produced.

#### THE 'EPIDEMIC CYCLE' IN MALARIA.

The following is a brief summary of the account given by Gill (1928) of the sequence of events comprising the epidemic cycle in malaria, as observed in the Punjab :—

The pre-epidemic period, which is that between the date of the appearance of the immediate determining cause (i.e., excessive rainfall) and the first increase in morbidity due to malaria, is characterized by the maintenance of a high degree of atmospheric humidity and a great numerical increase of the carrier species of anophelines.

The actual commencement of the epidemic is heralded by a sudden rise in the intensity of infections in the human host (average positive parasite count) followed, but at a slower rate, by an increase in the parasite rate. The rise in the parasite rate is closely followed by an increased morbidity, and slightly later by a corresponding rise in the spleen rate. The wave of increased mortality commences about one month later than that representing morbidity, and attains its maximum in from one to two weeks. The epidemic wave is accompanied by an increase in the infection rate among the carrier species of anophelines.

The parasite rate reaches its acme about the 12th week from the commencement of the epidemic, at the same time as does the spleen rate, and declines slowly but steadily thereafter. The average intensity of infections begins to decline earlier than this, i.e., while the frequency of infections is still rising. The spleen rate remains at or near its maximum until the end of the epidemic wave.

The wave of morbidity reaches its maximum about the 4th or 5th week of the epidemic, its decline being less abrupt than its rise. It does not regain its normal level till about 5 months from the commencement of the epidemic. The wave of mortality also declines more gradually than it has risen, but it reaches its normal level about the 12th or 13th week.

The numerical prevalence of the carrier species of anophelines remains high till about the end of the 4th week, after which the numbers decline, until, at about the 16th week, the species has almost completely disappeared.

The post-epidemic period is characterized during the first two years following the epidemic by enhanced waves of morbidity in the spring and autumn, whilst the birth rate in the year succeeding the epidemic is abnormally low. The parasite rate returns to normal in the course of two or three years, whilst the spleen rate, in spite of a small rise each autumn, declines gradually till it reaches its pre-epidemic level at the end of about 5 years in the case of major epidemics, though this period may be shorter in the case of epidemics of less severity.

The inter-epidemic period, during which the spleen and parasite rates remain at a low level, is of inconstant duration. A series of years of deficient rainfall, with or without famine, is sometimes followed by an abnormally heavy monsoon, and it is in these circumstances, which are apt to recur at irregular intervals, with some tendency to repetition every 10 years, that widespread major epidemics are prone to occur.

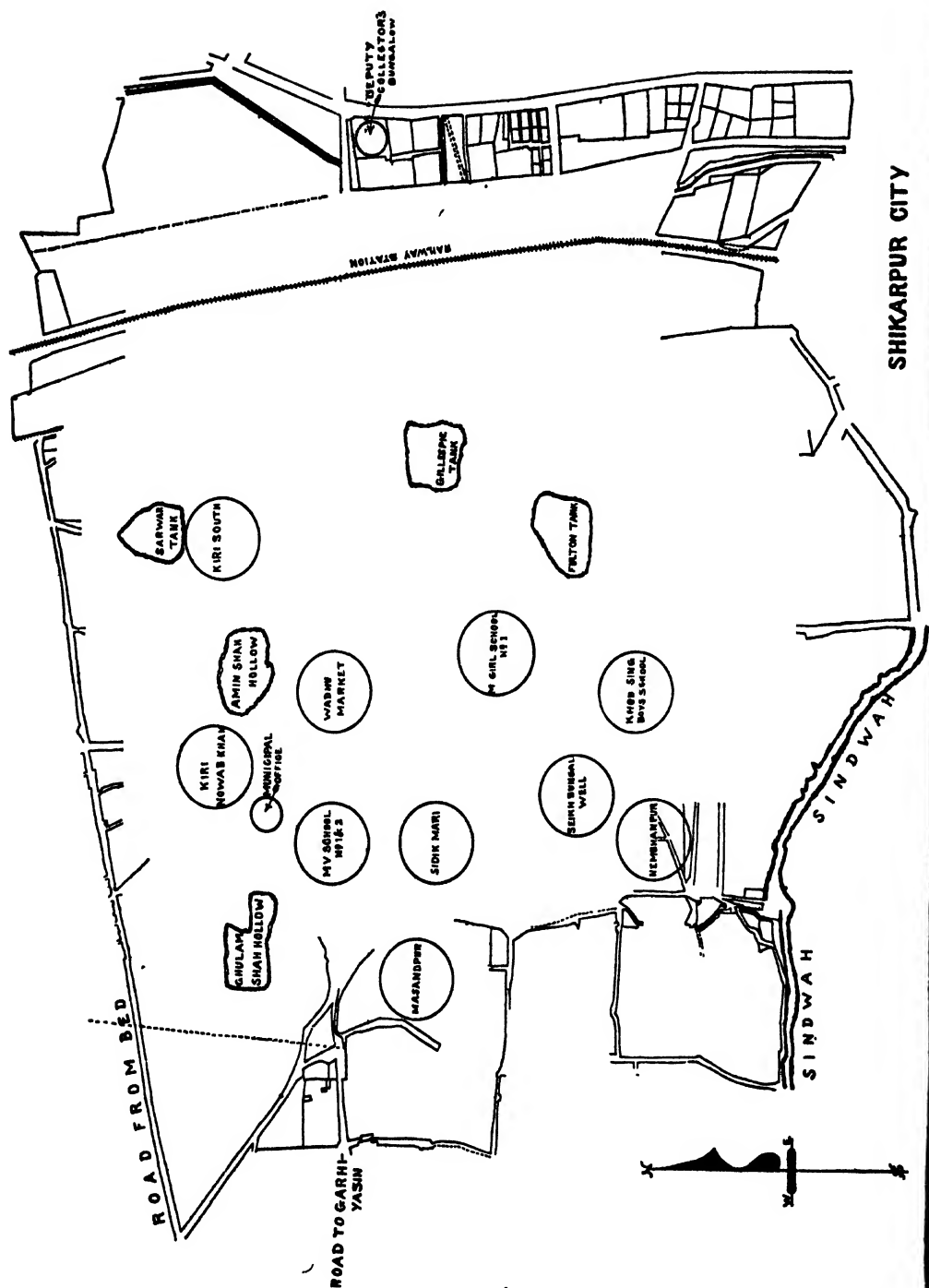
#### THE EPIDEMIC OF 1929 IN NORTHERN SIND.

##### Localities under observation.

Most of the observations here recorded were carried out in Shikarpur and in the villages in the immediate vicinity. This area was visited for the first time during the period August 27th to 31st, just as the epidemic was starting. Observations were made in certain villages in Ghotki, Ubauro and Mirpur Mithelo talukas of Sukkur District, which were also in the tract affected by the epidemic, during the last week in September. These villages are situated about 50 miles to the east of Shikarpur town. The course of the epidemic in Shikarpur was again closely studied from October 10th to November 6th, and further observations were made in this area during the last week in November and the third week in January.

Shikarpur (Map II), a town with 55,000 inhabitants, lies 20 miles to the north-west of Sukkur. The tract of country in which it is situated is low-lying, and the town itself is only 194 feet above the mean sea level. There are three large depressions or hollows, on the east, north-west and west aspects of the town respectively, into which flows the town sullage water and the excess irrigation water drained off from the surrounding fields and gardens. In years of abnormally heavy rainfall, such as that under review, these hollows are quite insufficient to deal with all the drainage of the town. The excess water is held up in the more low-lying portions of the town, with the result that after the heavy rainfall in 1929 the water was standing knee-deep round many of the buildings for a considerable period. The soil on which the town is built is relatively impervious, and myriads of pools were formed in and around the town, leading to a profusion of mosquito breeding.





### Inter-epidemic conditions.

No observations were made in Shikarpur itself previous to the epidemic, but a number of localities in the Upper Sind Frontier and Sukkur districts were visited during the years 1927 and 1928. The results of spleen examinations made in these districts (a) before the epidemic, and (b) in the year following the epidemic, are given in Table I.\*

As has been noted in a previous paper (Covell and Baily, 1931), the spleen rates recorded in Northern Sind prior to the epidemic were generally low, ranging for the most part from *nil* to 30 per cent, the higher figures being associated with increased local facilities for the breeding of *A. culicifacies*. The combined spleen rate for 1,180 observations made in Sukkur District in April 1927 was 11 per cent. The observations recorded in Pano Akil Taluka in October 1928 (i.e., during the malaria season) yielded somewhat higher figures, as was to be expected. The combined spleen rate for 446 observations made in the Upper Sind Frontier District in January 1928 was 21 per cent, and for 562 observations in November 1928 it was 28 per cent. \*

In the year following the epidemic it was found that the percentage of enlarged spleens throughout the affected area was extremely high, being in the great majority of instances over 75. The combined figure for 2,186 observations in the Upper Sind Frontier District was 81.0 per cent, whilst for 1,493 observations in Sukkur District it was 81.2 per cent.

### Pre-epidemic conditions.

As mentioned above, the year 1929 was marked by abnormally heavy rainfall in July and August throughout Sind. The daily amounts recorded at the headquarter towns of the talukas of Sukkur District are shown in Table II. The rainfall occurred during three periods, the first from July 12th to 16th, the second from July 25th to 30th, and the third from August 21st to 28th. No rain fell in Northern Sind during the month of September. In Shikarpur there were two excessively heavy falls, 4.08 inches being recorded on July 15th and 3.90 inches on August 26th. The monsoon rainfall recorded in Shikarpur for the years 1901—1930 is shown in Table III.

The existing canals of Sind are inundation canals, so constructed that they receive their full supply when the level of the river Indus as recorded at Bukkur Gauge reaches 12 feet. In 1929 this level was reached on July 23rd, and maintained continuously until September 11th. The river level during August was abnormally high, the daily average reading being 15.2 feet, as compared with an average of 12.6 feet for that month during the preceding 28 years. During the first 11 days of September the average reading was no less than 16.3 feet.

No records of the relative atmospheric humidity prevailing at Shikarpur during the pre-epidemic period are available, but the figures recorded at

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\* All the Tables referred to in the course of this paper will be found in the Appendix at the end.

Larkana, 40 miles to the south-west, show that the relative humidity in this locality in 1929 was approximately 10 per cent higher than during the corresponding period in 1928. The mean temperature, on the other hand, was about 5 degrees lower. It is permissible to assume that this condition of higher relative humidity and lower temperature prevailed throughout the epidemic area.

This period was also characterized at Larkana by a great increase in the numerical prevalence of anopheline mosquitoes, and especially of *A. culicifacies*, which has been shown, as the result of many observations carried out during the past five years, to be the principal, if not the sole, carrier of malaria in Sind (Table VIII).

#### The epidemic period.

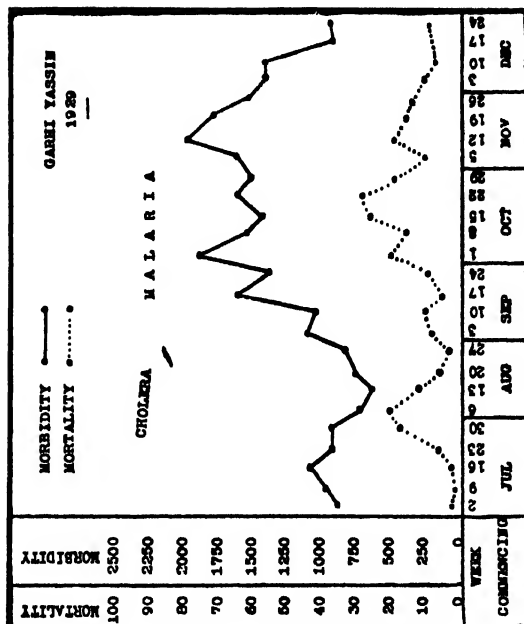
##### *Morbidity.*

The number of patients treated weekly for all diseases at the Civil Hospital, Shikarpur, during the years 1928 and 1929, are shown in Table IV, and those recorded during the period July to December in the latter year are represented in Graph I. There was no epidemic of any kind during the year 1928, and this may thus be looked upon as a normal year. It will be seen that the numbers of patients treated weekly showed but little variation, the highest number in any one week being 2,719 in the first week of December. In 1929 there was a rise in the number of cases treated at the end of July and first fortnight of August, on account of the cholera epidemic. The rise due to malaria began in the last week of August, when the number of cases rose from 2,131 to 3,323. The former figure is however abnormally low, the average number of cases treated each week being usually in the neighbourhood of 2,500. After a slight drop in the first week of September, the morbidity curve rose steadily till it reached its peak of 4,743 cases in the 6th week of the epidemic. There was then a steady drop to 3,500 in the 9th week. The figures rose to 3,716 in the 10th week, and thereafter showed a progressive decline, reaching the normal level in the 3rd week of December, i.e., the 16th week after the commencement of the epidemic.

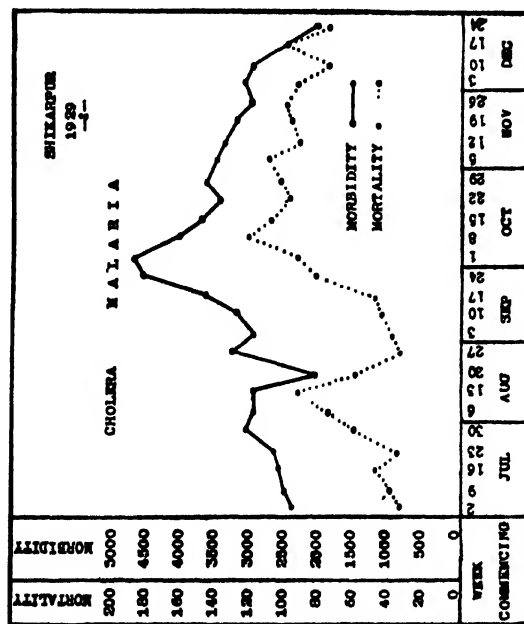
From the results of the examination of children during the epidemic, which are discussed in detail in a later section, it is deduced that the initial rise in the morbidity curve was due to a combination of benign tertian and malignant tertian infections. By the 6th week, when the morbidity figures reached their highest level, it is probable that almost every child was infected, the majority of the infections being with *P. falciparum*.

The second rise in the morbidity curve, which occurred in the 10th week of the epidemic, is considered to be due to relapses of malignant tertian infections. As will be seen later, there was a progressive decline in both the number and intensity of benign tertian infections after the 7th week, and there is no indication that relapses due to *P. vivax* occurred to any appreciable extent during the epidemic period.

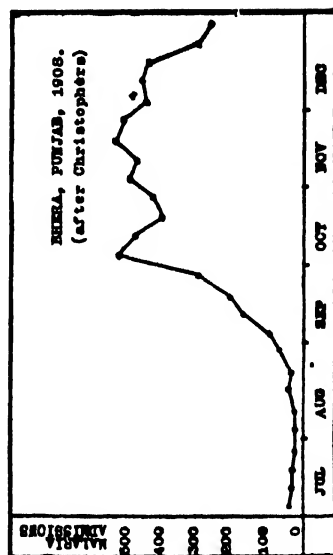
GRAPH II.



GRAPH I.



GRAPH III.



This second rise of the morbidity curve is also well shown in the case of Garhi Yassin, a town situated 11 miles south-west of Shikarpur (Table V and Graph II). It is interesting to note that the curve representing weekly attendances for malaria at Bhera (Punjab) in the case of the great 1908 epidemic (Christophers, 1911) also shows this character (Graph III), and bears a remarkable resemblance to the Garhi Yassin morbidity curve of 1929.

The exodus from Shikarpur City of a large proportion of the population on account of the cholera epidemic must have reduced the morbidity figures for the malaria epidemic period very considerably. It is impossible to say how many persons actually left the city, but the popular opinion was that they included approximately half the population. It is equally impossible to say when these people returned to their homes, but it is probable that the majority came back during the month of October.

#### *Mortality.*

The figures relating to mortality by weeks in Shikarpur City for the years 1928 and 1929 are given in Table VI and those recorded during the latter half of 1929 are also charted on Graph I. A sharp steep rise in the mortality curve, due to cholera, occurred in the first two weeks of August 1929. There was a slight progressive rise in the number of deaths from the second to the 4th weeks of the malaria epidemic, followed by a steep rise beginning in the 5th week and reaching its peak in the 7th week, i.e., one week after the highest peak of the morbidity curve. A second peak occurred in the 11th week, one week after the second rise previously noted in the case of the morbidity curve.

The common occurrence of a bimodal mortality curve in the course of malaria epidemics was pointed out by Christophers (1911). He showed that the first rise was attributable to the sudden mortality among infants and young children, and the second to the increasing number of deaths among adults, together with the continuance of a high death rate among children under 10 years of age.

In the present instance it will be seen from Table VI that the first peak in the mortality curve is due to a rise in the number of deaths in all classes, though the increase among infants is the chief factor. The deaths among persons over the age of 40 years continued to increase during the month of November, whilst the deaths amongst infants remained high, thus causing the second peak. In December the number of deaths amongst infants and children dropped considerably, those for the age period 10 to 40 years showed a slight rise, whilst the number of deaths among old people continued to rise, causing a third peak in the curve. It is probable that the progressive increase in the number of deaths amongst old people from October to December was due to the incidence of pneumonia and other respiratory diseases following on the debilitating effects of attacks of malaria.

An analysis of the age composition of the mortality in Shikarpur during the years 1928 and 1929 shows that the greatest *absolute* increase in the number

of deaths in the latter year occurred in the case of old people, and the next greatest in infants under two years. The greatest *relative* increase in mortality also occurred amongst the old people, but the next greatest was in the age group 2 to 10 years, particularly in the first half of that period. The remaining two groups showed an approximately equal relative increase over the figures recorded in 1928.

There was a considerable increase in the number of still-births recorded during the epidemic, the total number being 44 per cent greater than that during the corresponding period of 1928.

The remarks made in the last section as to the effect on the morbidity figures of the exodus of a large proportion of the population, apply also to the mortality figures.

#### *Prevalence of anopheline mosquitoes.*

It has been mentioned that the prevalence of anophelines, and especially of *A. culicifacies*, was unusually high in Larkana during the pre-epidemic period. Table VIII gives the percentage of this species to the total captures of adult anophelines in that area for the period July to December in the years 1928 to 1931.

Anopheline mosquitoes were abundant at Shikarpur at the commencement of the epidemic, and it is to be presumed that, as at Larkana, their numerical prevalence had been high throughout August. Out of 335 specimens captured at Shikarpur in the period August 27th to 31st, 50 per cent. were *A. subpictus*, 46 per cent *A. culicifacies*, 3 per cent *A. stephensi* and 1 per cent *A. pulcherrimus*.

The percentage of males of *A. culicifacies* caught was 43 at the commencement of the epidemic, whilst it had dropped to 6 by the seventh week, remaining at approximately this figure throughout the remainder of the epidemic period. It is inferred from this that the numbers of larvæ hatching out rapidly fell during the first few weeks of the epidemic, and that comparatively few adults were produced after the end of September.

#### *Breeding places of A. culicifacies.*

At the commencement of the epidemic, larvæ of *A. culicifacies* were found in profusion in Ghulam Shah and Amin Shah hollows, in irrigation wells and channels in gardens, in borrow-pits in brick-fields and elsewhere and in the numerous rain-water collections of all descriptions which were present in every quarter of the city.

By the beginning of October, all the temporary breeding places were dried up, but larvæ of this species were still present in the two hollows, and in certain irrigation wells and channels. During the month of November the only breeding-places of *A. culicifacies* discovered were the two hollows and a few borrow-pits in a brick-field.

*Natural infectivity of anophelines.*

The results of dissections carried out at Shikarpur and the neighbouring villages during the epidemic are shown in Table IX. The sporozoite rate among *A. culicifacies* was 1.9 per cent at the commencement of the epidemic (48 observations only), and it reached its peak of 30 per cent during the 10th week, i.e., one week later than the highest figure reached by the crescent rate: after which it declined to 6.5 per cent in the 13th to 14th weeks. It was noticed that during the decline of the epidemic the movement of the sporozoites found seemed to be abnormally sluggish.

The oöcyst rate was 13.3 per cent (45 observations only) at the commencement of the epidemic. It rose to 34 per cent in the 7th week, and was at the same figure in the 10th week. After this it declined, and was only 2 per cent in the 13th to 14th weeks. It was noted that the proportion of heavy gut infections, which was high during the early stages of the epidemic, became much less after the end of October, the average number of oöcysts found in infected guts being very small during the last few weeks of the epidemic.

*Spleen and blood examinations made in Shikarpur City during the epidemic period.*

A large number of observations were made by us in North Sind during the height of the epidemic and during the post-epidemic period, but it was only in Shikarpur City that spleen and blood examinations were carried out at the very commencement of the epidemic. At this period the spleen rate among 340 children (of which 205 were school children and the remainder street children) was less than 1 per cent, indicating that the communal immunity of the population against malaria when the epidemic began was practically *nil*. Further observations among the school children of Shikarpur were made in the 7th, 8th, 9th, 10th, 13th and 20th weeks of the epidemic. Though the number of observations made at each visit was comparatively small they are analysed here separately, because the figures obtained from the spleen and blood examinations represent the effects of a virulent epidemic of malaria upon a population which we know to have been previously non-immune.

In analysing the results it is necessary to keep in mind the fact that no observations were made in Shikarpur City during the six weeks which intervened between August 31st (first week of the epidemic) and October 10th, 1929. A second important point to remember is that the observations were made only on children who were sufficiently well to attend school. This remark applies to most if not all of the results which have been published in connection with epidemics of malaria. The virulence of the attacks is so great in this type of malaria, especially in the early stages of the epidemic, that a very large proportion of the children are prostrated, and are thus excluded from observation, whether the examinations are carried out in the schools or in the streets. A true picture of the epidemic could only be obtained by a house to house inspection, including the examination of all the sick, as well as of those who are

in a convalescent condition. As a rule it is not possible to carry out this procedure.

The results here discussed were obtained in three of the schools in Shikarpur City, viz., Khob Singh Boys' School, situated in the southern quarter of the City, the Municipal Vernacular Sindi Boys' School (M. V. S. School), situated in the northern quarter, and the Municipal Vernacular Hindu Girls' School (M. V. H. Girls' School), which is in the central portion. The various schools were closed at certain times during the epidemic, so that it was impossible to make observations in all of them at each visit. The results of the spleen and blood examinations carried out in these schools during the epidemic period are given in Table X and Graphs IV and V. As the results differed considerably in the different schools it is necessary to consider them separately.

*Khob Singh School.*—In the first week of the epidemic the spleen rate was 1 per cent and the parasite rate 21 per cent, the benign tertian rate being 7 per cent and the malignant tertian rate 14. No crescents were encountered. The spleen rate was 16 in the 7th week, and 23 per cent in the 8th week, but by the 20th week it had fallen to 9 per cent. Blood examinations in the 7th, 8th and 10th weeks gave the following results:—Parasite rate 78, 58, 74 per cent; benign tertian rate 14, 16, 10 per cent; malignant tertian rate 62, 41, 67 per cent; crescent rate, 2, 7, 52 per cent. The parasite rate in the 20th week was 31 per cent, the benign tertian rate being 1 per cent and the malignant tertian rate 30 per cent. The crescent rate at this period was 1 per cent.

*M. V. S. School.*—In the first week of the epidemic the spleen rate was 1.7 per cent and the parasite rate 27 per cent, the benign tertian rate being 12 and the malignant tertian 27. In the 9th week the spleen rate was 27 per cent and the parasite rate 76 (benign tertian rate 14, malignant tertian rate 69, crescent rate 54). In the 13th week the corresponding figures were: spleen rate 29, parasite rate 76 (benign tertian rate 2, malignant tertian rate 73, crescent rate 41). No further observations were made in this school during the epidemic period.

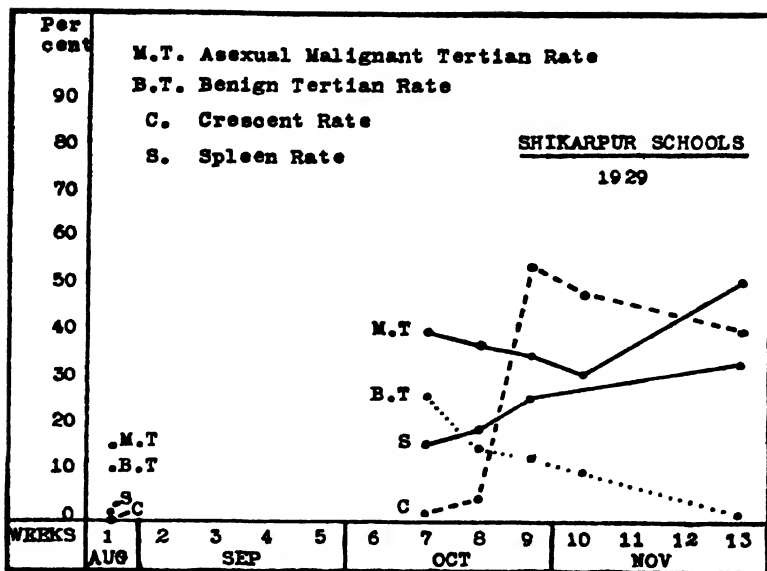
*M. V. H. Girls' School.*—This school was closed at the commencement of the epidemic, and was not visited until the 7th week. Blood examinations made in the 7th, 8th and 10th weeks gave the following results:—parasite rate 60, 60, 75; benign tertian rate 40, 17, 16; malignant tertian rate 22, 43, 66; crescent rate 2, 2, 25. The spleen rate was 19 in the 7th week and 15 in the 8th week (an insignificant difference), but it had risen to 46 per cent in the 13th week.

The value of the above-quoted figures is discounted to some extent by the facts that the numbers of observations were small, and that none were made in Shikarpur City between the 1st and 7th weeks of the epidemic period. Nevertheless, certain points of interest emerge from a study of the figures.

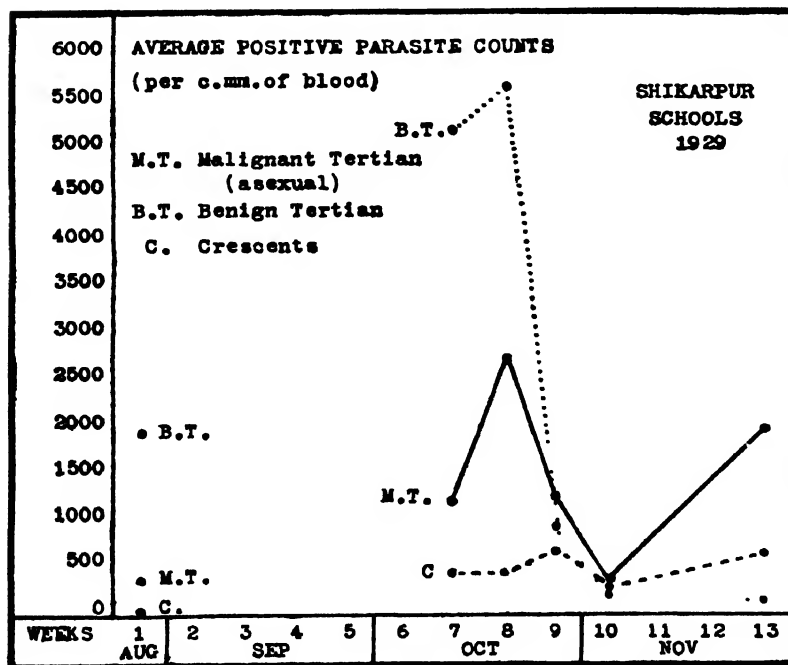
A striking feature in the blood examinations is the sudden rise in the crescent rate occurring about the 9th week of the epidemic period, i.e., 3 weeks after the highest peak of the morbidity curve. In the case of Khob Singh School the crescent rate rose from 2 per cent and 7 per cent in the 7th and 8th



GRAPH IV.



GRAPH V.



weeks respectively to 52 per cent in the 10th week. In the M. V. S. School, where no crescents were encountered in the first week of the epidemic, the rate was 54 per cent in the 9th week. In the M. V. H. Girls' School the crescent rate was 2 per cent in both the 7th and 8th weeks and 25 per cent in the 10th week.

The highest benign tertian rate observed in Khob Singh and M. V. S. schools was 16 per cent, whereas in the M. V. H. Girls' School the rate was 40 per cent in the 7th week, falling to 17 per cent one week later. It is of course possible (and indeed probable) that the benign tertian rate in the other two schools had been at a higher level during the six weeks period in which no observations were made (1st to 7th weeks).

Taking the figures for the three schools together, it will be seen that the benign tertian infections showed a steady fall, both in number and in intensity, from the 7th week onwards. It appears that there were few if any relapses due to *P. vivax* during the latter 2 months of the epidemic period. If the figures relating to the asexual malignant tertian parasites are studied, it will be seen that here also there was a fall in the number and intensity of infections from the 7th to the 10th weeks, but that there was a marked rise in both in the 13th week. It is considered that this indicates that a large number of the malignant tertian infections relapsed during the latter part of the epidemic period.

In all the schools the high parasite rate during the epidemic period, as contrasted with the comparatively low spleen rate, was a marked feature.

The size of the average enlarged spleen during the epidemic period varied considerably on the different dates on which the examinations were carried out, and showed an increase in size from 10 to 8.2 cm. (apex-umbilicus measurement) between the 8th and 13th weeks.

The percentage frequencies of the different sizes of spleens on different dates are also of considerable interest. Covell and Baily (1927) showed that the size of the average enlarged spleen in chronic malaria was considerably higher than in acute malaria, though the spleen rate might be equally high in both cases. Christophers (1929), referring to these results, said that there appeared to be two kinds of enlarged spleen, the spleen of acute malaria, with a mean apex-umbilicus measurement of about 10 cm., and the spleen of immune malaria, with its mean nearer the umbilicus. Macdonald (1931) discussed the bimodal character commonly present in the frequency polygons representing the various sizes of enlarged spleen in malaria, and was of opinion that the first peak in the curve represents acute infections, whilst the second is the effect of acute infections superimposed upon chronic infections, i.e., infections of two years or more duration.

In the present instance we are dealing with a population in which the spleen rate was less than 2 per cent at the commencement of the epidemic, and therefore practically all the enlarged spleens subsequently encountered must represent acute infections. It will be seen from Graphs VI and VII that the curve was unimodal in the 7th week, but that it afterwards became bimodal,

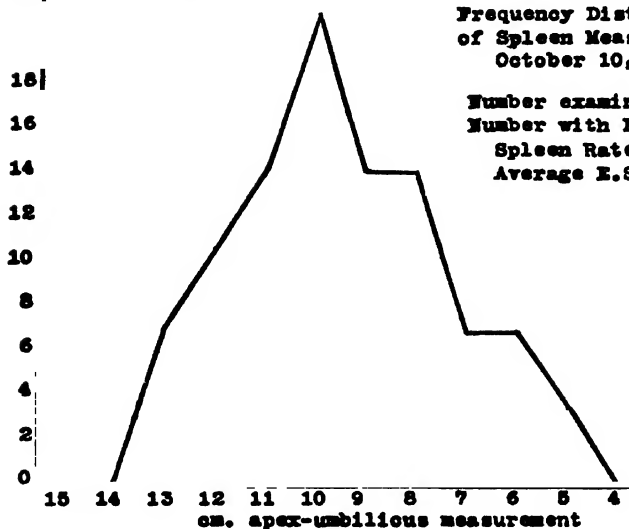
GRAPH VI.

Percentage of all  
enlarged spleens  
22,

SHIKARPUR SCHOOLS

Frequency Distribution  
of Spleen Measurements  
October 10, 1929

Number examined 161  
Number with E.S. 28  
Spleen Rate 17.4%  
Average E.S. 9.2 cm.



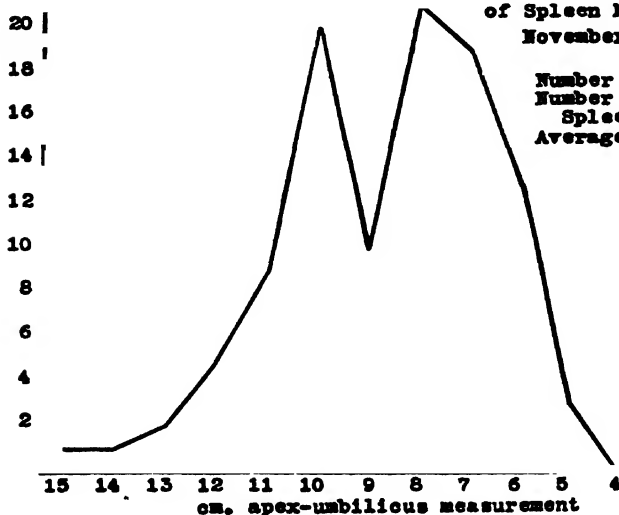
GRAPH VII

Percentage of all  
enlarged spleens  
22,

SHIKARPUR SCHOOLS

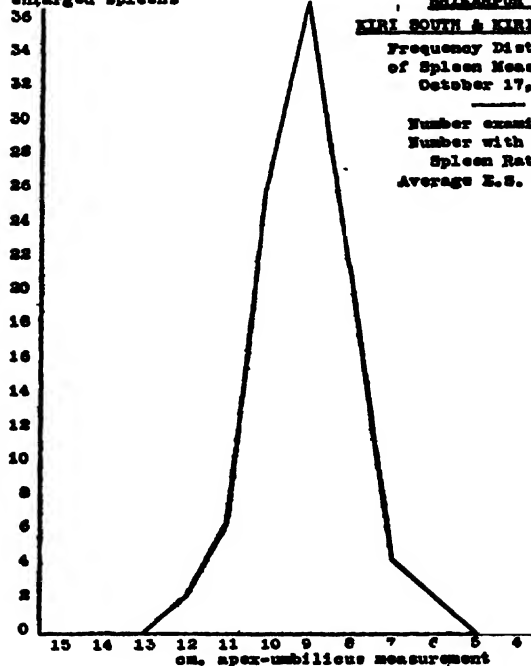
Frequency Distribution  
of Spleen Measurements  
November 25, 1929

Number examined 310  
Number with E.S. 105  
Spleen Rate 34%  
Average E.S. 8.8 cm.



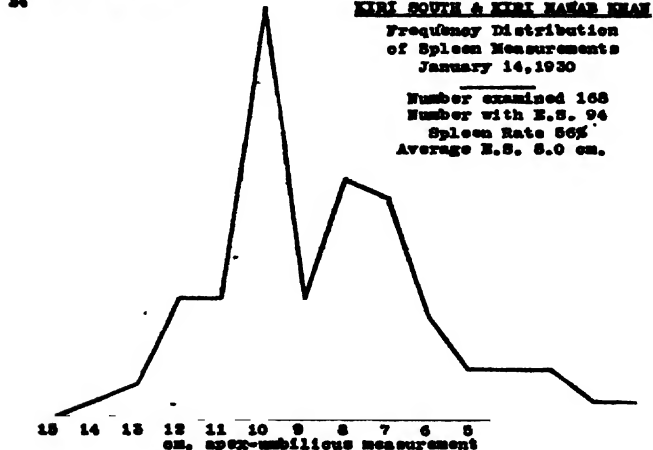
GRAPH VIII.

Percentage of all  
enlarged spleens



GRAPH IX.

Percentage of all  
enlarged spleens



the second peak becoming more and more pronounced until by the 13th week it had become slightly higher than the first. The first peak represents spleens of 10 cm. apex-umbilicus measurement, and the second spleens of 8 and 7 cm. measurement. This change in the character of the curve, taking place in the course of a few weeks, shows that there were two classes of spleen present during the acute stage of the epidemic. As has been stated above, the results of blood examinations indicate that there were a large number of relapses of malignant tertian infections during the latter part of the epidemic period, and it seems probable that the larger spleens, forming the second peak in the curve, are the result of these relapses. Had they been caused by fresh infections, we should have expected also a considerable increase in the number of smaller spleens, together with a marked rise in the spleen rate.

The fact that the spleen rate during the epidemic period remained comparatively low in spite of the very high parasite rate indicates either that there were a large number of infected children in which the spleen did not become palpable, or, more probably, that there were a large number of cases in which the spleen became acutely engorged but rapidly subsided in size.

An even more pronounced change in the curve representing the percentage frequencies of the different sizes of spleen is shown in the case of street children in the northern quarter of the City (Kiri South and Kiri Nawab Khan). These observations were made in the 8th and 20th weeks of the epidemic, and the results are set out in Graphs VIII and IX. The spleen rate in each case was practically the same (53 per cent and 56 per cent). The size of the average enlarged spleen showed a slight increase at the second examination, from 8.7 cm. to 8.0 cm. apex-umbilical measurement. The curve representing the different sizes of spleen is unimodal in the first instance with the peak at 9 cm., whilst in the second case it is markedly bimodal, with peaks at 10 cm. and 8 cm. No observations were made in this quarter at the commencement of the epidemic, but the character of the first curve strongly suggests that the enlarged spleens were practically all acute infections, and that the spleen rate before the epidemic had been as low as that in the schools.

The fact that the spleen rate in this quarter had reached a figure of over 50 per cent by the 8th week, whilst that in the schools at this period was only 20 per cent, indicates that the infections commenced earlier at the periphery of the City (which is nearer to the breeding places of *A. culicifacies*) and subsequently spread to the more central portion. The conditions found in Kiri South and Kiri Nawab Khan closely resemble those in the surrounding villages. The observations relating to the latter will be dealt with in a later paper.

Results of observation made in the first eight months of the post-epidemic period.

No observations were made in Shikarpur between January 1930 and August 1930. The results of spleen and blood examinations made in the last

*Malaria Epidemic in Northern Sind.*

GRAPH X.

Percentage of all  
enlarged spleens

20

18

16

14

12

10

8

6

4

2

15 14 13 12 11 10 9 8 7 6 5 4  
cm. apex-umbilicus measurement

SHIKARPUR SCHOOLS

Frequency Distribution  
of Spleen Measurements  
August 26, 1930

Number examined 414  
Number with E.S. 187  
Spleen Rate 45%  
Average E.S. 9.8 cm.

GRAPH XI.

Percentage of all  
enlarged spleens

20

18

16

14

12

10

8

6

4

2

0

15 14 13 12 11 10 9 8 7 6 5 4  
cm. apex-umbilicus measurement

SHIKARPUR CITYKIRI SOUTH & KIRI HAWAB KHAN

Frequency Distribution  
of Spleen Measurements  
August 29, 1930

Number examined 85  
Number with E.S. 73  
Spleen Rate 86%  
Average E.S. 8.2 cm.

week of the latter month are given in Tables X and XI and Graphs X and XI. The most striking features shown by these figures as compared with those obtained at the end of the epidemic period are (1) a marked increase in the spleen rate, (2) a decrease in the size of the average enlarged spleen, (3) a great decrease in the malignant tertian parasite rate, and (4) a marked increase in the benign tertian parasite rate.

These changes in the spleen and blood pictures occurring during the first eight months immediately following the epidemic period were not confined to Shikarpur, but were equally marked in the other places in North Sind where observations were carried out.

In the case of the school children examined in Shikarpur in August 1930, the benign tertian parasite rate, which had been very low indeed during the last part of the epidemic period (13th week 2 per cent, 20th week 1 per cent), was now 19 per cent; whereas the malignant tertian rate, which had been 62 per cent in the 9th week, 63 per cent in the 10th, 73 per cent in the 13th and 29 per cent in the 20th, was now only 5 per cent. The spleen rate in the M. V. H. Girls' School was somewhat lower than it had been in the 13th week of the epidemic, but in the case of each of the other two schools the spleen rate was more than twice as great as it had been at any visit made during the epidemic period. The average enlarged spleen had decreased in size in all the schools.

The preponderance of benign tertian infections during the months immediately succeeding an epidemic has been recorded by many authors, notably by Christophers (1911) in the case of observations made in the Punjab in the spring following the great epidemic of 1908. It has been supposed that this was due to the greater persistence in the body of the benign tertian parasite. But in the present instance it has been shown that benign tertian infections, as far as could be detected by blood examinations, practically disappeared during the latter part of the epidemic period, though they had been fairly common in the first few weeks.

We consider that the increase in the spleen rate found throughout Northern Sind eight months after the end of the epidemic was the result of attacks of benign tertian malaria occurring in the spring and early summer. These can scarcely have been caused by new infections acquired during this period, owing to the almost complete absence of the carrier species of *Anopheles*, and to the generally unfavourable meteorological conditions for the transmission of malaria. They may have been either delayed primary attacks resulting from infections received during the previous autumn, or delayed relapses ('recurrences') of attacks which occurred in the early weeks of the epidemic. The fact that the size of the average enlarged spleen was everywhere decreased suggests that the majority of the attacks were of the former nature. Had they been chiefly relapses, we would have expected an increase, instead of a decrease, in the size of the average enlarged spleen.

It may be objected that the increase in the benign tertian parasite rate might be due to fresh infections with *P. vivax*, denoting the advent of the autumn malaria season of 1930. Had this been so we would have expected a high intensity of infections, whereas the average positive benign tertian count in August 1930 was only 384 per c.mm. of blood (for 26 cases in the M. V. S. School it was less than 200), whilst in August 1929 the corresponding figure had been 1,950 (Table X). Furthermore, we would not have expected a marked rise in the spleen rate at this early stage.

Korteweg (1902) concluded that the cases of malaria occurring in Holland in the spring were the result of primary infections acquired during the previous autumn. Plehn (1919) remarks that latent infections with benign tertian malaria reveal themselves in the spring, and Sinton (1931) records that a considerable number of latent primary infections with *P. vivax* amongst British troops were detected at Kasauli (under conditions which precluded the possibility of fresh infections) several months after the subjects had arrived there. Swellengrebel (1921, 1922, 1924) has shown that malarial infection among anophelines in Holland is practically absent during the spring, when malaria is rife. Schüffner, Korteweg and Swellengrebel (1929) experimentally infected six persons with *P. vivax* in the autumn of 1928 by the bites of one or two mosquitoes in each case. None of these persons showed any sign of malaria either clinically or parasitically until from 34 to 38 weeks after exposure to infection, but every one of them had an attack of benign tertian malaria at some period between the 8th and 10th months.

James (1931) records a series of 12 cases of experimental infections with *P. vivax* in paretics in which the primary attack occurred between 7 and 11 months after infection, and remarks 'There would be many more cases of this type in our records if, in the practice of malariatherapy, it was not the rule that the clinical malarial attack must be brought on with as little delay as possible'. In most cases, when clinical symptoms fail to appear at an early date, re-inoculation either by mosquito bites or by blood must be repeated until an attack is produced.

As regards relapses of benign tertian malaria occurring at intervals of more than 26 weeks from the date of the primary infection (which he terms 'recurrences'), James records 31 cases in which this was observed among 107 patients. Anderson (1922) records that relapses amongst the troops at Salonika were frequent in the spring and summer, but rare in the winter, when the climatic conditions were most severe, a fact which was also observed in Macedonia and Turkey by the senior author of the present paper.

James is of opinion, at least as regards Northern Europe, that the 'spring rise' in malaria cases is due to recurrences in persons who have had their primary attack in September, together with primary attacks in persons whose infections in September remained latent through the winter. He further concludes that in natural circumstances the occurrence of these cases in the spring is not due to any special climatic or environmental conditions peculiar to that



season, but results from the fact that April and May are between 30 and 40 weeks after September, when the primary infections were received.

It appears to us that James' hypothesis is the most likely explanation of what occurred in North Sind during the spring and summer months following the autumn epidemic of 1929.

We may summarize the probable course of events as follows:—In the early part of the epidemic period there were a large number of infections both with *P. vivax* and with *P. falciparum*, but a very considerable proportion of the former remained latent during the epidemic period. It is possible that these were persons who had received only a comparatively small dose of sporozoites. During the latter half of the epidemic period a large proportion of the malignant tertian infections relapsed, but few if any benign tertian relapses occurred during this period. In the spring and early summer the latent infections with *P. vivax* occasioned numerous attacks of malaria, and probably there were also a number of late relapses ('recurrences') in those who had had attacks of benign tertian malaria in the early autumn.

#### DISCUSSION.

The conditions in Northern Sind in 1929 were peculiarly favourable for the occurrence of an outbreak of fulminant epidemic malaria. No major epidemic had occurred since 1917, so that the majority of the child population below the age of 12 years had probably seldom or never been exposed to infection. The level of communal immunity against malaria was thus at a very low point, a fact that was confirmed by the generally low level of the spleen rate.

A series of years of deficient rainfall had preceded the year of the epidemic, whilst the excess of rainfall in July and August, and the occurrence of widespread floods due to the combined effects of rainfall and of the overflow of canals and rivers, created a state of sustained high atmospheric humidity in the pre-epidemic period. An epidemic of cholera, combined with the effects of the floods which resulted in widespread destruction of property and cattle, had caused a large number of people to abandon their homes, with consequent dislocation of trade and economic distress.

The epidemic itself in most respects followed the characteristic course described by Christophers and by Gill in the case of malaria epidemics in the Punjab. As the result of the creation of numerous breeding places by the excessive rainfall, and the high degree of relative atmospheric humidity, which rendered conditions especially favourable to mosquito life, there was an abnormal increase in the numerical prevalence of anophelines, and especially of *A. culicifacies*, the chief malaria carrier of the province, in the pre-epidemic period.

The intensity of infections among the child population was high during the early stages of the epidemic. The parasite rate and spleen rate and the waves of morbidity and mortality behaved in the usual manner, although the parasite rate reached its highest peak rather earlier than is described by Gill. There

was a very high rate of natural infections of *A. culicifacies* during the epidemic. Finally, there was a marked decrease in the birth rate in the year following the epidemic.

The appearance during the course of the epidemic period of a second mode in the curve representing the frequencies of different sizes of spleen indicates that there were, even at this stage, two different types of acutely enlarged spleen amongst the population. As has been already stated, we consider that the second peak in the curve is due to the occurrences of early relapses of malignant tertian malaria. This hypothesis is supported by the increase in both the number and intensity of asexual malignant tertian infections during the latter half of the epidemic period, following a drop in both these figures at the end of the second month, and also by an increase in the size of the average enlarged spleen. The larger spleens cannot have been the result of acute infections superimposed upon chronic infections, because the spleen rate at the commencement of the epidemic was less than 2 per cent.

The marked increase in the spleen rate which occurred during the first eight months of the post-epidemic period, associated with a decrease in the size of the average enlarged spleen, has not been recorded in the case of any other malaria epidemic, so far as we are aware. As has been stated above, we consider that this phenomenon, accompanied as it was by a marked increase in the number of *P. vivax* infections, which had been very few indeed during the latter half of the epidemic period, was due to the occurrence of a large number of acute attacks of benign tertian malaria during the spring and early summer; and that these attacks were caused by delayed primary infections with *P. vivax* acquired in the previous autumn, together with a certain number of late relapses ('recurrences') of the same species of parasite.

A striking feature of the blood picture was the paucity of crescents at the commencement of the epidemic. Unfortunately, no blood examinations were made until the last four days of August, and at that period only 88 blood films were examined. In none of these were crescents found, and it may be argued from this that at any rate crescent carriers among the population must have been very few at this stage.

This is by no means an isolated observation, for many other workers have remarked upon the extreme scarcity of gametocyte carriers before, and at the commencement of, an epidemic of malaria. Caccini (1902) stated that several observers in Italy had noted the absence of crescents when the new malaria season was imminent, and that he himself had never found crescents in that country from April to June. Coulon and Sautet (1931) found no crescents in Corsica from May to July, though they were present from August to April, being most numerous in November and December. MacGilchrist (1915) found no crescents among the prisoners in a jail in Bengal in September, though they were present in October, November and December. Sinton (1926), working among the prisoners in Lahore Central Jail, Punjab, found crescents in only scanty numbers in the period July to October, though they were present in

much greater numbers in November and December. Macdonald and Majid (1931) record the paucity of crescent carriers, both before and at the beginning of the malaria season, as being one of the outstanding features of their observations in the Punjab. Ross (1911) and Ziemann (1914) have suggested that the malaria parasite may have a tendency to produce gametocytes at a period just previous to the maximum prevalence of the mosquito carrier. But, as Sinton has pointed out, if these suggestions were correct, we should expect the rise in the incidence of crescent carriers to occur several weeks earlier than is actually the case.

Reference has already been made to the sudden rise in the crescent rate which was observed to occur amongst the school children in Shikarpur at about the 9th week of the epidemic period. The combined figures for two of the schools showed a rise in the number of crescent carriers from 5 to 49 per cent between the 8th and 10th weeks, whilst in the case of the third school, in which no crescents had been detected in the first week, there was a crescent rate of 54 per cent in the 9th week. Sinton (1926) commented on the sudden rise of the number of crescent carriers amongst the prisoners in Lahore Jail from one per cent in October to ten per cent in November.

As was pointed out by Macdonald and Majid (1931), approximately one month must elapse between the day on which an anopheline has fed on a crescent carrier and the day on which crescents can appear in the peripheral blood of a person infected by that anopheline. If we assume, for purposes of argument, that one crescent carrier, under optimum conditions for transmission, can give rise to ten crescent carriers after the lapse of one month, this would explain an increase in the number of crescent carriers from 5 per cent in the 5th week of an epidemic to 50 per cent in the 9th week. Further, in order to produce a crescent rate of 5 per cent in the 5th week, it would only be necessary for the number of crescent carriers to be one in 200 in the first week of the epidemic. If this theory is correct, we would also expect that the crescent rate would increase by sudden rises at intervals of approximately one month, as long as all the other conditions favourable to transmission remained unchanged.

We think that this manner of approaching the problem helps to explain why a paucity of crescent carriers in the pre-epidemic period need not be incompatible with the occurrence of a severe epidemic of malaria, provided that the period during which conditions are pre-eminently favourable for transmission is sufficiently prolonged. In most cases where the results of blood examinations made during the period immediately preceding the malaria season have been published, the number of observations has been comparatively small, and moreover the thin film method has generally been used. It can easily be understood that, where the crescent rate is of the order of one per 200 persons, the presence of crescent carriers might be missed altogether, and we consider that this is the explanation of the negative findings which have frequently been recorded.

It was concluded by James (1926), as the result of certain laboratory observations on *A. maculipennis* in England, that infected anophelines usually pass their whole life in the house where the infection was acquired. Investigations by many other workers in different countries have however shown that there is normally a very considerable to and fro movement of anophelines in and out of buildings, and that even shortly after taking a blood meal anophelines may change their abode (Roubaud, 1920; Barber and Hayne, 1924; Davis, 1926; Missiroli and Hackett, 1927; Kligler and Liebman, 1928; Boyd, 1930; Richmond and Mendis, 1930). It seems to us that there must necessarily be a very considerable amount of dispersion of *A. culicifacies* from the houses in which they have fed to explain the spread of infections originating from the small number of gametocyte carriers normally present among the population immediately before the commencement of the malaria season.

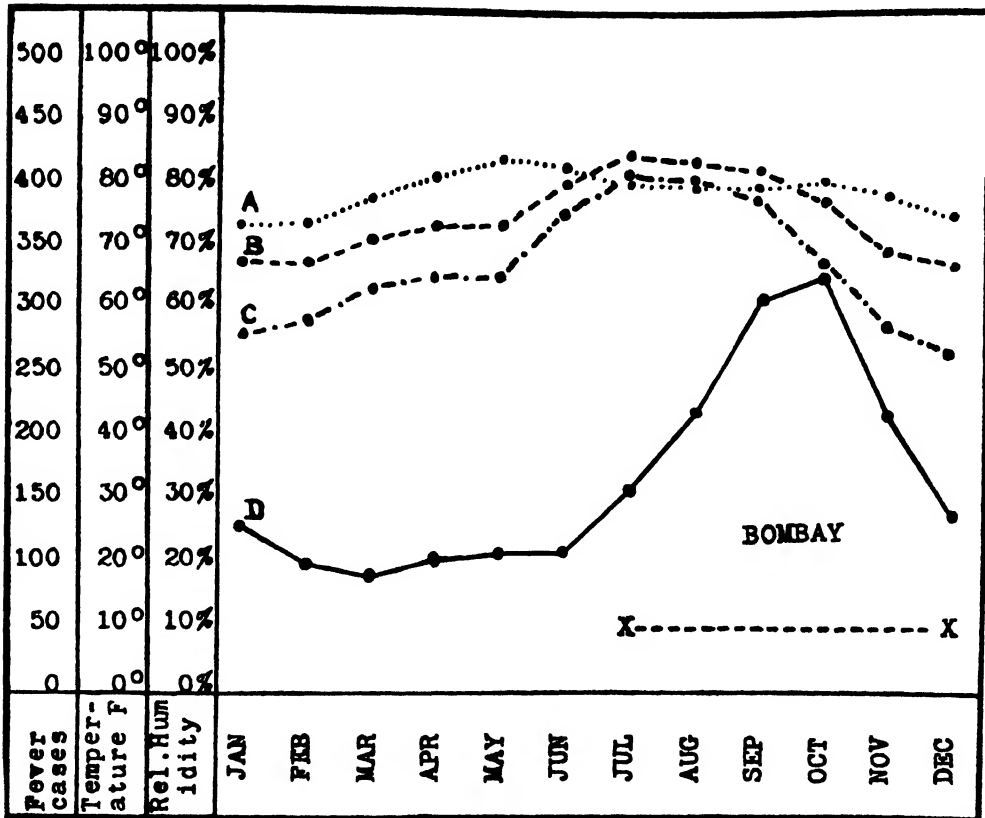
Gill (1921) has shown that the lower limits of temperature and relative atmospheric humidity favourable for the transmission of malaria in the Punjab are represented approximately by a mean monthly temperature of 61°F., and a mean monthly relative humidity (8 a.m. reading) of 63 per cent. It does not follow, however, that whenever these figures are exceeded the transmission of malaria will be actively carried on in a community. There is evidence to show that the *optimum* conditions for transmission are represented by a considerably higher degree of relative humidity than the figure given above.

Mayne (1928) dissected over 2,000 specimens of *A. culicifacies* under natural conditions at Saharanpur, United Provinces, but did not encounter any infected specimens till the weekly mean humidity exceeded 80 per cent. with temperatures of 75° to 83°F. Covell and Baily (1930) did not find any infected specimens of *A. culicifacies* at Larkana, Sind, before the beginning of August, when the mean relative humidity rose to over 70 per cent. Bentley (1911), in Bombay, found infected specimens of *A. stephensi* from July to December, but none during the period January to June, although the mean relative humidity in Bombay is over 70 per cent from March onwards. Covell (1928), in Bombay, obtained similar results, no infected mosquitoes being found till July 10th, when the mean relative humidity exceeded 80 per cent (Graph XII). Hartman (1928), as the result of his observations in South China, concluded that the optimum condition for the mosquito transmission of malaria was represented by an average relative humidity of 70 to 80 per cent, together with a temperature of between 65° and 82°F. Necheles (1925) concluded that a relative humidity of 75 to 80 per cent is the optimum for *Anopheles*. Senior White, as the result of field observations, is also of the opinion that in India malaria transmission does not commence until a very high degree of humidity has been reached (Knowles and Senior White, 1930).

Mayné (1930) showed that different species of anophelines react differently to the effects of temperature and humidity, and that in this respect *A. culicifacies* is more sensitive than *A. fuliginosus* and *A. subpictus* to adverse meteorological conditions.

We are of opinion that the period during which the active transmission of malaria takes place in Northern Sind is normally not more than 6 weeks in length, but that this may be increased by about 4 weeks in years when excessive rainfall occurs in the monsoon period; and that it is the increase in the length of the period of high sustained relative atmospheric humidity thereby produced which is the main factor in the production of an epidemic of malaria. It is

GRAPH XII.



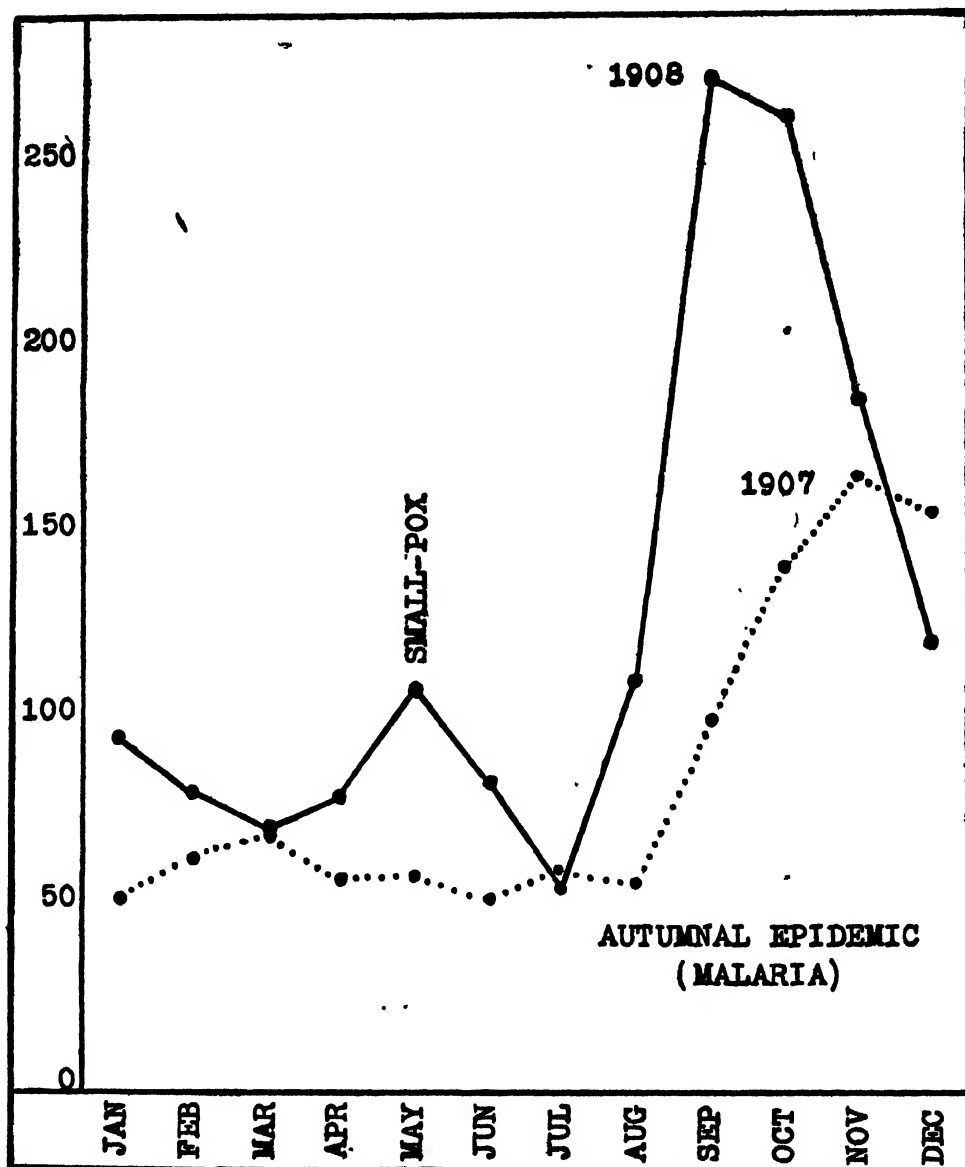
A Mean temperature. B Mean relative humidity. C Relative humidity at 2 p.m. D Number of outpatients treated for malaria at the Port Trust Dispensary. X-----X Months in which *A. stephensi* has been found naturally infected.

noteworthy that in the case of the great malaria epidemic in the Punjab in 1908, the autumnal epidemic rise in the mortality curve commenced one month earlier than in 1907 (Graph XIII).

The results of our observations seem to afford certain indications as regards the control of malaria in tracts subject to regional epidemics.

The importance of engineering operations designed to minimize the effects of flooding, caused either directly by excessive rainfall or indirectly by the

GRAPH XIII.



Graph showing the monthly mortality at Shahpur, Punjab, for the years 1907 and 1908 (after Christophers, 1911).

overflow of canals or rivers, is obvious. It is equally apparent that adequate arrangements should be made for a sufficient supply of quinine to be available in areas which are threatened by an epidemic; i.e., where several years of deficient rainfall and low fever incidence have been succeeded by an abnormally heavy monsoon.

As regards anti-larval operations of a temporary nature, the lesson seems to be that every effort should be concentrated against the breeding places of the carrier species during the pre-epidemic period and the first few weeks of the epidemic. In the Punjab and Northern Sind this would be roughly from the beginning of July to the end of September. It is doubtful if anti-larval measures carried out after the end of September in these areas are of any great value, since the great majority of the anophelines concerned in the production of the epidemic have been hatched out before that date.

With regard to the mass administration of plasmoquine or other drugs with the object of rendering gametocyte carriers non-infective to anophelines, it is clear that the arrangements for the distribution of the drug during the pre-epidemic period must be very complete if the scheme is to succeed, since an epidemic of malaria may occur even though the percentage of carriers among the population during that period has been very small indeed.

#### SUMMARY

1. A regional, or fulminant, epidemic of malaria occurred in Northern Sind in the autumn of 1929. The results of observations carried out in Shikarpur immediately before the epidemic, during the epidemic period, and during the first eight months of the post-epidemic period, are given in detail.

2. The precipitating cause of the epidemic was excessive monsoon rainfall accompanied by flooding, following a series of years in which rainfall had been in defect. The relative atmospheric humidity was abnormally high during the pre-epidemic period.

3. There had been no major epidemic in Sind since 1917. The amount of communal immunity against malaria, as indicated by the results of spleen examinations, was generally at a very low level.

4. During the epidemic period the spleen rate, parasite rate, intensity of infections, morbidity rate and mortality rate behaved for the most part in the manner characteristic of epidemics which have previously been observed in the Punjab.

5. *P. falciparum* was the principal malaria parasite concerned in the epidemic, and was responsible for most of the mortality. A number of relapses due to this parasite occurred during the latter part of the epidemic period. In a large proportion of cases any enlargement of the spleen which may have resulted from infection with this species of parasite very rapidly subsided.

6. There was an abrupt rise in the crescent rate about the 9th week of the epidemic period, i.e., 2 or 3 weeks after the highest peak of the morbidity curve.

7. During the course of the epidemic period the curve representing the frequencies of different sizes of spleen, which was at first unimodal in character, became markedly bimodal. It is considered that the larger spleens were the result of early relapses of malignant tertian infections. This hypothesis is supported by the fact that the size of the average enlarged spleen increased during this period, and that there was also a marked increase in the number and intensity of malignant tertian infections, following a drop in both these figures which had been observed between the 7th and 10th weeks.

8. In the early stages of the epidemic period there were a number of cases of benign tertian malaria. There was no indication that these relapsed during the epidemic period.

9. During the first eight months of the post-epidemic period there was a marked increase in the spleen rate, associated with a decrease in the size of the average enlarged spleen, and a considerable increase in the benign tertian parasite rate, which had been very low during the latter half of the epidemic period. It is considered that this indicates that a number of delayed primary attacks of benign tertian malaria, resulting from infections acquired in the previous autumn, occurred during this period, together with probably a certain number of delayed relapses ('recurrences') due to the same species of parasite.

10. The numerical prevalence of *A. culicifacies* was high at the commencement of the epidemic, but there is evidence that very few adults of this species were hatched out after the first few weeks.

11. There was a high infectivity rate amongst *A. culicifacies* during the epidemic, the sporozoite rate reaching 30 per cent at one period. During the decline of epidemic it was noted that the sporozoites encountered were abnormally sluggish.

12. During the year following the epidemic the birth rate in the affected area was abnormally low. Spleen rates in rural areas were everywhere raised to approximately 80 per cent or higher.

13. The results of the observations are discussed with reference to the mechanism of causation of regional epidemics of malaria, and with regard to their bearing on the application of control measures.

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## APPENDIX.

TABLE I.

*Results of spleen examinations in Northern Sind in (a) the Inter-epidemic period and (b) the Post-epidemic period.*

LOCALITY.	INTER-EPIDEMIC PERIOD			POST-EPIDEMIC PERIOD.		
	Date.	Number examined.	Splenic index.	Date.	Number examined.	Splenic index.
<b>KASHMOR TALUKA.</b>		UPPER SIND FRONTIER DISTRICT.				
Kashmor ..	i. 28	62	11·2	x. 30	111	82·0
Gulam Mohd	i. 28	35	11·4	x. 30	30	73·3
<b>KANDHKOT TALUKA.</b>						
Kandhkot ..	i. 28	92	28·0	ix. 30	201	84·0
	xi. 28	204	10·0			
Haybat ..	i. 28	39	28·5	ix. 30	58	88·0
	xi. 28	70	5·7			
Dari ..	xi. 28	110	20·9	ix. 30	100	92·0
Ghauspur ..	xi. 28	178	62·9	ix. 30	120	91·7
<b>THUL TALUKA.</b>						
Mir Hasan ..	i. 28	27	33·0	ix. 30	53	83·0
Thul ..	i. 28	66	24·0	ix. 30	181	87·3
<b>JACOBABAD TALUKA.</b>						
Jacobabad ..	i. 28	100	17·0	x. 30	347	64·6
Jatohi ..	i. 28	25	32·0	x. 30	55	89·0
<b>SUKKUR TALUKA.</b>		SUKKUR DISTRICT.				
Punjabi ..	iv. 27	25	20·0	x. 30	29	93·0
Rahuja ..	iv. 27	22	4·5	x. 30	41	88·0
Abad ..	iv. 27	40	9·0	x. 30	104	85·6
Arain ..	iv. 27	62	8·0	x. 30	63	85·7
Shahpur ..	iv. 27	15	0·0	x. 30	24	95·8
Old Sukkur ..	iv. 27	207	22·2	x. 30	270	80·4

TABLE I—*concl'd.*

LOCALITY.	INTER-EPIDEMIC PERIOD.			POST-EPIDEMIC PERIOD.		
	Date.	Number examined.	Splenic index.	Date.	Number examined.	Splenic index.
ROHRI TALUKA.	SUKKUR DISTRICT— <i>concl'd.</i>					
Miani ..	iv. 27	26	38.5	x. 30	48	91.5
Bichanji ..	iv. 27	128	28.1	x. 30	60	86.7
Kasim ..	iv. 27	106	4.7	x. 30	60	83.3
Jahan Khan	iv. 27	31	17.9	x. 30	52	90.4
Gosarji ..	iv. 27	25	0.0	x. 30	90	78.9
Lakhi ..	iv. 27	103	3.9	x. 30	150	92.7
Begargi ..	iv. 27	87	5.7	x. 30	121	76.0
Dodo Kot ..	iv. 27	25	8.0	x. 30	24	87.5
Kandra ..	iv. 27	160	2.5	x. 30	112	59.0
PANO AKIL TALUKA.						
Pano Akil ..	x. 28	120	12.5	x. 30	70	67.7
Pitalfi ..	x. 28	20	10.0	x. 30	21	57.1
Uri Jani Jo ..	x. 28	61	27.9	x. 30	41	90.2
Shahpur ..	x. 28	44	27.3	x. 30	10	70.0
Sadhuja ..	x. 28	36	25.0	x. 30	75	91.0
Sawai Kalwar	x. 28	52	25.0	x. 28	28	68.0

TABLE II.

*Daily amounts of rainfall in inches recorded at the headquarter towns of four talukas in Sukkur District during July and August 1929.*

Day of month.	SHIKARPUR.		GHOTKI.		UBAORO.		MIRPUR MITHILO.	
	July.	August.	July.	August.	July.	August.	July.	August.
1	..	0.28	..	..	..	..	..	..
2	..	..	..	..	..	..	..	..
3	..	..	..	..	..	..	..	..
4	..	..	..	..	..	..	..	..

TABLE II.—concl'd.

Day of month.	SHIKARPUR.		GHOTKI.		UBAURO.		MIRPUR MITHILO.	
	July.	August.	July.	August.	July.	August.	July.	August.
5	..	..	0'25	..	1'02	..	0'49	..
6	..	..	..	..	..	..	..	..
7	..	..	..	..	..	..	..	..
8	..	..	..	..	..	..	..	..
9	..	..	..	..	..	..	..	..
10	..	..	..	..	..	..	..	..
11	..	..	..	..	..	..	..	..
12	..	..	0'51	..	0'24	..	0'04	..
13	0'10	..	..	..	..	..	0'02	..
14	0'33	..	2'00	..	0'56	..	1'60	..
15	4'08	..	..	..	0'09	..	..	..
16	0'06	..	..	..	..	..	..	..
17	..	..	..	..	..	..	..	..
18	..	..	..	..	..	..	..	..
19	..	..	..	..	..	..	..	..
20	..	..	..	..	..	..	..	..
21	..	..	..	..	..	1'50	..	0'58
22	..	1'08	..	0'79	..	0'17	..	..
23	..	0'27	..	0'20	..	..	..	..
24	..	..	..	..	..	0'20	..	0'12
25	0'65	0'80	..	0'75	0'05	0'02	0'31	0'28
26	..	3'90	0'14	0'32	1'98	..	1'99	1'17
27	0'65	0'08	0'80	0'07	0'05	..	0'35	0'35
28	0'27	..	0'10	0'97	..	..	..	..
29	..	..	..	..	0'22	..	..	..
30	0'02	..	0'07	..	..	..	0'16	..
31	..	..	..	..	..	..	..	..
TOTAL	5'56	6'41	3'87	3'10	4'21	1'89	4'96	2'50

N.B.—No rainfall was recorded at any of the above stations during the month of September 1929.

TABLE III.

*Monthly rainfall figures in inches recorded at Shikarpur during the period  
July-September for the years 1901-1930.*

Year.				July.	August.	September.	REMARKS.
1901	..	..	..	1'71	..	..	
1902	..	..	..	..	0'25	3'00	
1903	..	..	..	..	..	..	
1904	..	..	..	..	..	..	
1905	..	\	..	..	..	..	
1906	..	..	..	..	3'93	..	Epidemic year
1907	..	..	..	0'11	1'71	..	
1908	..	..	..	0'63	2'47	..	
1909	..	..	..	0'37	..	..	
1910	..	..	..	1'50	0'10	..	
1911	..	..	..	..	0'04	..	
1912	..	..	..	0'10	0'04	0'50	
1913	..	..	..	1'54	3'31	0'35	
1914	..	..	..	2'25	..	..	
1915	..	..	..	..	..	0'19	
1916	..	..	..	0'28	0'77	..	
1917	..	..	..	..	4'36	8'75	Epidemic year.
1918	..	..	..	..	..	0'02	
1919	..	..	..	0'41	0'31	..	
1920	..	..	..	0'09	..	..	
1921	..	..	..	0'07	0'26	0'43	
1922	..	..	..	..	..	..	
1923	..	..	..	0'07	3'14	..	
1924	..	..	..	0'06	..	0'30	
1925	..	..	..	2'10	1'73	..	
1926	..	..	..	..	1'84	0'20	
1927	..	..	..	0'64	..	..	
1928	..	..	..	0'22	0'12	..	
1929	..	..	..	5'56	6'41	..	Epidemic year.
1930	..	..	..	2'93	..	..	

TABLE IV.

*Total number of patients treated weekly at the Civil Hospital, Shikarpur, during the years 1928 and 1929.*

Week commencing			1928.	1929.	Week commencing			1928.	1929.
January	1	..	2,456	2,612	July	2	..	2,630	2,489
"	8	..	2,600	2,611	"	9	..	2,687	2,524
"	15	..	2,585	2,670	"	16	..	2,451	2,678
"	22	..	2,453	2,623	"	23	..	2,465	2,715
"	29	..	2,670	2,422	"	30	..	2,452	3,132
February	5	..	2,625	2,429	August	6	..	2,673	3,006
"	12	..	2,483	2,554	"	13	..	2,608	2,996
"	19	..	2,185	2,529	"	20	..	2,526	2,131
"	26	..	2,213	2,489	"	27	..	2,496	3,323
March	5	..	2,651	2,439	September	3	..	2,612	3,000
"	12	..	2,604	2,082	"	10	..	2,588	3,273
"	19	..	2,443	2,066	"	17	..	2,754	3,712
"	26	..	2,592	2,207	"	24	..	2,691	4,613
April	2	..	2,572	2,299	October	1	..	2,641	4,743
"	9	..	2,527	2,269	"	8	..	2,562	4,107
"	16	..	2,615	2,253	"	15	..	2,618	3,756
"	23	..	2,578	2,266	"	22	..	2,683	3,500
"	30	..	2,546	2,326	"	29	..	2,688	3,716
May	7	..	2,652	2,341	November	5	..	2,605	3,550
"	14	..	2,583	2,218	"	12	..	2,650	3,450
"	21	..	2,479	2,171	"	19	..	2,455	3,286
"	28	..	2,303	2,282	"	26	..	2,426	3,039
June	4	..	2,572	2,382	December	3	..	2,719	3,169
"	11	..	2,569	2,285	"	10	..	2,597	3,076
"	18	..	2,533	2,333	"	17	..	2,617	2,517
"	25	..	2,521	2,444	"	24	..	2,613	2,115

TABLE V.

*Total number of patients treated weekly at the Civil Hospital, Garhi Yassin, during the years 1928 and 1929.*

Week commencing			1928.	1929.	Week commencing			1928.	1929.
January	1	..	638	641	July	2	..	671	919
"	8	..	546	591	"	9	..	722	991
"	15	\ ..	579	591	"	16	..	659	1,108
"	22	..	480	642	"	23	..	701	965
"	29	..	521	650	"	30	..	560	969
February	5	..	568	595	August	6	..	782	734
"	12	..	710	564	"	13	..	663	664
"	19	..	592	611	"	20	..	599	774
"	26	..	675	552	"	27	..	715	869
March	5	..	605	612	September	3	..	593	1,123
"	12	..	620	596	"	10	..	730	1,084
"	19	..	612	627	"	17	..	695	1,684
"	26	..	618	650	"	24	..	702	1,601
April	2	..	561	675	October	1	..	776	1,933
"	9	..	597	648	"	8	..	691	1,571
"	16	..	578	614	"	15	..	748	1,479
"	23	..	539	624	"	22	..	699	1,672
"	30	..	558	675	"	29	..	596	1,546
May	7	..	600	622	November	5	..	746	1,702
"	14	..	551	630	"	12	..	691	2,012
"	21	..	530	700	"	19	..	659	1,868
"	28	..	552	676	"	26	..	630	1,575
June	4	..	559	722	December	3	..	629	1,452
"	11	..	664	803	"	10	..	583	1,455
"	18	..	715	720	"	17	..	584	967
"	25	..	679	802	"	24	..	570	980



TABLE VI.

*Total number of deaths recorded weekly from July to December at Shikarpur, during the years 1928 and 1929.*

Week commencing			Total deaths, 1928.	Total deaths, 1929.	AGE COMPOSITION OF MORTALITY, 1929.				
					Still-births.	0-2 years.	3-10 years.	11-40 years.	41 years and over.
July	2	..	20	35	1	11	10	6	7
"	9	..	25	40	1	15	7	3	14
"	16	..	35	49	4	13	10	11	11
"	23	..	38	35	1	7	12	9	6
"	30	.	25	61	3	24	12	13	9
August	6	..	26	77	3	19	21	22	12
"	13	..	23	94	1	25	24	23	21
"	20	..	31	60	1	17	11	17	14
"	27	..	28	35	2	13	6	7	7
September	3	..	26	39	3	13	9	5	9
"	10	..	26	45	3	20	6	5	11
"	17	..	26	49	7	19	5	9	9
"	24	..	39	84	12	30	12	13	17
October	1	..	26	94	6	31	9	15	33
"	8	..	32	123	8	44	15	19	37
"	15	..	47	115	4	37	18	29	27
"	22	..	30	99	7	29	16	22	25
"	29	..	41	104	6	45	15	14	24
November	5	..	31	111	10	39	6	15	41
"	12	..	29	93	6	34	12	16	25
"	19	..	52	98	10	43	5	12	28
"	26	..	42	101	12	27	10	22	30
December	3	..	37	95	7	30	8	23	27
"	10	..	34	77	7	24	4	8	34
"	17	..	47	103	8	30	5	49	41
"	24	..	42	77	10	20	2	12	33

TABLE VII.

*Total number of deaths recorded weekly from July to December at Garhi Yassin, during the years 1928 and 1929.*

Week commencing.	Total deaths, 1928.	Total deaths, 1929.	AGE COMPOSITION OF MORTALITY, 1929.			
			0-2 years.	3-10 years.	11-40 years.	41 years and over.
July 2 ..	4	3	2	0	1	0
" 9 ..	1	2	0	0	2	0
" 16 ..	1	3	1	1	1	0
" 23 ..	6	7	1	4	2	0
" 30 ..	1	18	4	6	2	6
August 6 ..	4	21	2	7	4	8
" 13 ..	0	13	2	3	6	2
" 20 ..	4	7	2	1	3	1
" 27 ..	2	4	1	1	1	1
September 3 ..	4	9	8	0	1	0
" 10 ..	2	11	7	0	1	3
" 17 ..	3	6	4	1	0	1
" 24 ..	3	10	9	1	0	0
October 1 ..	3	21	11	5	1	4
" 8 ..	4	17	9	2	3	3
" 15 ..	5	27	16	6	1	4
" 22 ..	2	29	21	5	0	3
" 29 ..	4	20	10	5	3	2
November 5 ..	2	11	7	1	0	3
" 12 ..	6	20	14	1	2	3
" 19 ..	5	17	6	3	4	4
" 26 ..	3	15	7	3	2	3
December 3 ..	3	12	4	2	2	4
" 10 ..	3	8	3	2	0	3
" 17 ..	4	9	3	0	3	3
" 24 ..	2	10	4	2	1	3

TABLE VIII.

*Percentage of A. culicifacies to total number of anophelines captured in villages near Larkana during the period July to December in the years 1928-1931.*

Month.				1928.	1929.	1930.	1931.
July	..	..	..	1	6	No observations.	
August	..	..	..	5	16	4	1
September	..	..	..	4	40	14	7
October	..	..	..	34	35	16	28
November	..	..	..	37	46	26	55
December	..	..	..	78	No observations.	57	63

TABLE IX.

*Results of dissections of A. culicifacies, August-November 1929.*

Date	MID-GUT.			SALIVARY GLANDS.			TOTAL.		
	Number examined.	Number infected.	Oöcyst rate.	Number examined.	Number infected.	Sporozoite rate.	Number examined.	Number infected.	Infection rate.
August 27th-31st.	45	6	13·3	52	1	1·9	52	6	11·5
September 24th-30th	74	7	9·5	74	4	5·4	74	10	13·5
October 11th-16th.	47	16	34·0	49	8	16·3	49	20	40·8
17th-22nd	94	27	28·7	96	12	12·5	97	27	27·8
23rd-30th.	71	15	21·1	84	12	14·3	84	20	23·8
October 31st- November 2nd	50	17	34·0	50	15	30·0	50	23	46·0
November 8th-14th.	127	27	21·3	130	17	13·1	135	34	25·2
15th-25th.	105	3	2·9	108	7	6·5	108	9	8·3
TOTAL ..	613	118	19·2	643	76	11·8	649	149	24·5

*Note.*—The specimens dissected in September were caught in certain villages in Ghotki, Ubauro and Mirpur Mithelo talukas of Sukkur District. All the other specimens dissected were caught in Shikarpur and in the neighbouring villages

TABLE X.  
Results of spleen and blood examinations made in Shikarpur schools, August 1929-August 1930.

Date.	Name of school.	Number examined for enlarged spleen.	Percentage found with enlarged spleen.	A U measurement of average enlarged spleen.	Number of bloods examined.	Percentage with parasites.	Average positive parasite count per c mm. of blood.	Percentage with M. T. parasites.	Average positive M. T. parasite count per c mm.	Percentage with asexual M. T. parasites.	Average positive asexual M. T. count per c mm.	Percentage with crescent.	Average positive crescent count per c mm.	Percentage with B. T. parasites.	Average positive B. T. parasite count per c mm.
1929. 27-viii	Khob Singh	90	11	..	14	21	..	14	..	..	..	..	..	7	..
	M. V. S. School	115	17	..	74	27	..	16	..	..	..	..	..	12	..
	TOTAL ..	205	15	11.3	88	27	1,025	16	364	16	364	..	..	11	1,950
10-x	Khob Singh	88	16	..	50	78	..	62	..	..	..	1	..	14	..
	M. V. H. Girls'	73	19	..	50	60	..	22	..	..	..	1	..	40	..
	TOTAL ..	161	17	9.2	100	69	2,715	42	1,212	41	1,191	2	429	27	5,214
17-x	Khob Singh	83	23	..	58	55	..	41	..	..	..	7	..	15	..
	M. V. H. Girls'	81	15	..	42	59	..	43	..	..	..	2	..	17	..
	TOTAL ..	164	20	10.0	100	57	3,431	42	2,719	38	2,808	5	428	16	5,698
24-x	M. V. S. School	180	27	9.0	99	76	1,403	69	1,247	36	1,090	55	692	14	960

29-i	..	Khob Singh	.	..	..	88	74	..	67	..	..	52	..	10	..
		M. V. H. Girls'	..	.	.	12	73	..	66	..	..	25	..	16	..
		TOTAL	..	..	.	100	74	348	67	365	32	247	49	11	100
25-ii	..	M. V. S. School	231	29	..	90	76	..	73	..	..	41	..	2	..
		M. V. H. Girls'	79	47	.	.	..	..	..	..	..	..	..	..	..
		TOTAL	310	34	8-8	90	76	1,922	73	1,977	52	2,284	41	2	110
1930.															
15-i	..	Khob Singh	92	9	9-4	75	31	412	29	427	29	424	1	80	80
26-viii	..	Khob Singh	121	31	10-5	40	17	..	3	.	..	..	..	13	..
		M. V. H. Girls'	120	36	10-2	30	23	.	0	..	..	..	..	13	..
		M. V. S. School	173	61	9-4	120	28	..	7	..	..	..	..	22	..
		TOTAL	414	45	9-8	180	24	377	5	351	..	..	0-6	19	384

TABLE XI.

*Results of spleen examinations in the northern quarter of Shikarpur City  
(Kiri South and Kiri Nawab Khan).*

Date.	Number of children examined.	Number found with enlarged spleen.	Spleen rate.	Apex-umbilicus measurement of average enlarged spleen (cm.).
October 10th, 1929	89	47	53	87
January 14th, 1930	168	94	56	80
August 29th, 1930	85	73	86	82

TABLE XII.

*Results of spleen examinations at Garhi Yassin and certain villages in the neighbourhood of Shikarpur in January and August 1930.*

Locality.	Month	Number examined	Number found with enlarged spleen	Spleen rate	Apex-umbilicus measurement of enlarged spleen (cm.).
Garhi Yassin ..	January	270	133	49	83
	August	337	206	65	90
Madeji ..	January	80	49	61	81
	August	129	104	81	76
Dhakkan ..	January	72	56	78	67
	August	82	71	81	72
Bed, Kamman and Khakipotah.	January	155	40	71	75
	August	133	110	83	90

## STUDIES IN IMMUNITY IN MALARIA.

### Part I.

#### *AN INTRADERMAL REACTION IN MALARIAL INFECTIONS IN MONKEYS.*

BY

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#### INTRODUCTION.

THE unsuccessful results of all attempts to infect any of the common laboratory animals with human malaria have been in the past a very serious handicap to experimental work in this disease. Although much information has been gathered from the researches into avian malaria, there are many disadvantages and limitations in such work and also in the interpretation of the results as applied to malaria in mammals.

The discovery of the therapeutic value of malarial infection in general paralysis of the insane has opened up a wide field for invaluable experimental research, but even here the fact that infections cannot be allowed to run their natural course, in many instances, restricts the usefulness of this line of investigation.

Few or any of the common laboratory mammals have been found naturally infected with *Plasmodia*, except the monkey. Many workers in past years have recorded infections in various species of these animals. Although in some instances the strain seemed easily transmissible to the common brown monkey, *Silenus* (*Macacus*) *rhesus*, yet few of the observers seem to have used the monkey parasite as a laboratory strain for the investigation of malarial problems in recent years. This was possibly on account of the cost of such

animals in many places and partly because of the difficulties in keeping the strain going. Research work on the infection in such animals under suitable conditions should lead to important results.

Napier and Campbell (1932), during the course of routine examinations of the blood of laboratory monkeys, discovered an infection with *Plasmodium* sp. in *Cercopithecus pygerythrus*. This parasite was found to cause a very acute infection, when injected into the common brown monkey of India, *Silenus* (*Macacus*) *rhesus*. Such infections were sometimes followed by fatal results in spite of quinine treatment, and in some cases the infection was complicated by hæmoglobinuria.

Knowles and Das Gupta (1932) have given a description of this parasite as observed in its original host, as well as in induced infections in *S. (M.) rhesus* and some other genera and species of monkey. They have also succeeded in causing an infection in human volunteers. Extreme differences in the susceptibilities of hosts of different genera and species of monkey were accompanied by very considerable variability in parasite morphology. For this reason it was not found possible to identify the parasite definitely with any of the previously described species of *Plasmodium* in monkeys.

We are deeply indebted to Lieut.-Colonel R. Knowles, I.M.S., Professor of Protozoology, and Lieut.-Colonel H. W. Acton, C.I.E., I.M.S., Director of the School of Tropical Medicine, Calcutta, for giving us the strain referred to above. This strain has been used for most of the experiments recorded in this paper. Recently, however, we have obtained another monkey *Plasmodium*, morphologically resembling *P. cynomolgi*, but with a 24-hour, not a 48-hour, cycle. We hope to carry out additional work with this strain. This parasite was discovered in a specimen of *S. (M.) pileatus* (Blyth), (*Pithecus pileatus*) (Blyth)† examined by us. It has been found capable of producing infections in *S. (M.) rhesus*, when injected subcutaneously.

Now that two strains of parasite are available, it should be possible to carry out various comparative studies to determine whether any special findings are peculiar to one strain as compared with the other.

There seems to be at present only one absolutely certain method for the diagnosis of active malaria infection and that is by the discovery of the malarial parasite. This method may not be possible, however, under all conditions and the absence of some other reliable diagnostic method has long been felt. Many workers have made unsuccessful attempts to evolve another practical and satisfactory test to overcome this difficulty.

Our work commenced as an attempt to discover whether any serological reaction could be devised, which would prove useful either in the detection of

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\* Our sincere thanks are due to Dr. Bains Prashad, D.Sc., F.R.S.E., Officiating Director of the Zoological Survey of India, for the identification of these monkeys.

† While this paper was in the press Dr. Bains Prashad has informed us that these monkeys has been found, on further examination, to be immature specimens of *Silenus irus* (*Macacus cynomolgus*), and not *S. pileatus* as formerly intimated.



malarial infection or of the changes produced by such infection. Researches into the precipitin tests described by Taliaferro, Taliaferro and Fisher (1927), Taliaferro and Taliaferro (1928) and by Row (1931) gave no uniform results in our hands. 'Antigens'\* were therefore prepared in various other ways to investigate this and other serological reactions.

One of us (J. A. S.) had previously made unsuccessful attempts in 1923 to obtain a skin reaction with parasites obtained from cultures of *P. falciparum*. It was thought that the failure of these attempts was possibly due to the small amount of parasite material available, and that larger amounts might give more satisfactory results. It was hoped at the same time that some indication might be obtained as to the diagnostic potentialities of the antigens prepared by different methods. Intradermal injections of various antigens were tried on infected monkeys and a comparison made with the results obtained in normal animals. There was found to be a very striking difference between the reactions obtained in these animals, when an 'antigen' prepared by the digestion of malarial parasites with papain was used.

A preliminary note recording the results of this intradermal reaction has already been published (Sinton and Mulligan, 1932) and it is proposed in the present paper to give fuller details of methods used and the results obtained.

#### PREPARATION OF ANTIGENS

The most successful antigen, so far used, was prepared by digesting with papain a suspension of malarial parasites which had been freed from blood cells.

It had been found during the course of our investigations that, when citrated blood from an infected monkey was centrifuged, the parasitized cells tended to collect as a thick brownish or greyish-brown layer overlying the uninfected red blood corpuscles. A somewhat similar observation had been made by Bass and Johns (1915) in connection with *P. falciparum* in human blood. It was further noticed that the parasite layer was larger and the separation from the non-infected corpuscles was more complete, when the blood contained many parasites approaching the mature stage of schizogony. It was found possible by repeated use of the centrifuge to obtain as much as 15 ccs of compact deposit, composed almost entirely of parasitized red blood cells.

#### Isolation of malarial parasites in bulk

Induced malarial infection in *S. (M.) m. rhesus* was allowed to proceed unchecked until the parasite count was very high (sometimes as many as

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\* The term 'antigen' is not used in this article in the strict sense defined by Wells (1928) i.e., 'a substance which incites the development of specific reactive agents "antibodies", when introduced under proper conditions into the circulation or tissues of an animal'. It has, however, been found to be a convenient term to designate the product of the malarial parasite which has been employed for the tests described later. These products may possibly be of the nature of the 'residue antigens' of Zinsser and Mueller (1928) but until more work has been carried out to discover their nature, 'antigen' seems a convenient term to use.

2,500,000 per c.mm.) and it was anticipated that the animal would not survive another segmentation of the parasites. By frequent blood examinations the time when the parasites were near maturity was determined, and the infected blood was then collected.

The animal was anæsthetized with chloroform and the thorax opened before the heart stopped beating. Several 20 c.c. syringes, each charged with about 4 c.c. of citrate-saline solution, were used to withdraw the blood from the heart, and in this way it was possible to obtain about 80 c.c. blood from a small monkey.

The citrated blood was centrifugalized to separate the plasma from the cells. The former was pipetted off and the cells were afterwards washed twice in citrate-saline solution to get rid of all traces of plasma. The dark parasite layers were then removed from the different centrifuge tubes and concentrated again by renewed centrifugalization in saline solution. A deposit was eventually obtained which appeared to consist almost entirely, on both macroscopical and microscopical examinations, of parasitized cells.\*

The parasite mass was then mixed with distilled water to hæmolyse any remains of red cells present. By repeated washing with distilled water and centrifugalization, a dark grey deposit of parasites was obtained with a clear supernatant fluid free from hæmoglobin. This final deposit consisted practically entirely of free parasites and a few leucocytes. Few red cell stromata were present. These were more difficult to centrifugalize down than the heavier parasites and were in consequence mostly removed with the hæmoglobin-stained washing fluid.

#### *Digestion of parasites with papain.*

The parasite mass was washed out of the centrifuge tubes with double the amount of distilled water. A small amount of papain was added to the parasite suspension and the whole incubated in a water bath at about 60°C. for 4 hours, the mixture being repeatedly shaken the while. At the end of this time the digest was filtered through filter paper and a clear transparent fluid of a pale amber colour obtained. This filtrate was stored in the ice-chest and was used for intradermal tests as required. Some brews were found to be too concentrated and in consequence had to be diluted before use (*vide infra*).

Other antigens were prepared by papain digestion of some of the organs of infected monkeys, such as the liver, spleen and bone-marrow. In the case of solid organs, it was found convenient to grind them up with powdered glass in a mortar and suspend the paste in double its volume of distilled water before commencing digestion.

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\*In the earlier work the blood was filtered through sterile cotton-wool to remove as many of the white blood cells as possible, but in later work these were chiefly eliminated by pipetting off the 'buffy layer' which collected at different times on the surface of the centrifugalized deposit.

As far as possible aseptic precautions were taken during all the steps of the preparation of the different antigens.

*Sterilization of antigens.*

Although the filtrate from the papain digest proved to be a very active antigen for intradermal reactions, the possibility of contamination during the various stages of its preparation could not be overlooked. Some of the antigens, even when stored in the ice-chest, developed moulds after a few weeks. Attempts were made at sterilization by boiling on three successive days, but this rendered the antigen inactive.

It was found, however, that a sterile but active antigen could be obtained by adding the filtrate to an excess of absolute alcohol (about 10 times its volume). This resulted in the immediate formation of a copious yellowish or whitish precipitate, which was collected by centrifugalization and was found to be mostly resolvable in normal saline solution. Preparations made in this way under aseptic conditions, were found to remain sterile for long periods and to be as active, or even more so than, the original filtrates. The precipitates were in most cases allowed to remain in contact with the alcohol for 24-48 hours, but even longer periods did not seem to have any marked effect on their potency.

The addition of 0.5 per cent carbolic acid to the original filtrate has been tried. This seems to prevent bacterial growth and to have no serious effects on the potency of the antigen or harmful effects in normal monkeys. Whether prolonged exposure to carbolic will eventually affect its properties will require further observation.

*Standardization and dosage of antigens.*

As might be expected in a technique of preparation, in which it is impossible to make more than approximate measurements of the material used and in which no previous experience was available, the final products showed some difference in their relative potency. Whether these variations were caused by technical differences in preparation or were due to variations in the stage of development of the parasites used, it is impossible to say. Some antigens caused a very severe necrotic reaction in infected monkeys and even some redness and oedema in normal ones, while with some others the reaction in infected animals was comparatively mild. These differences seemed to depend mainly upon the proportion of parasite material to fluid in the suspension before digestion, but also to some extent upon the time allowed for digestion. The method of preparation described above seemed, however, to give a fairly uniform result.

The method used for the sterilization of antigens by alcohol precipitation also afforded an easy method for regulating the strength of the antigen. Thus if the reaction, either in the normal or infected animal, was found to be too severe, the precipitate from alcohol was redissolved in a quantity of saline

solution proportionately greater than the original volume of the antigen before precipitation. In a similar manner a weak antigen could be concentrated by solution of the precipitate in a smaller amount of saline solution.

By these means it was possible to obtain antigens of a strength which, while producing nothing except a slight immediate reaction in normal monkeys, yet caused a marked delayed one in infected animals.

In the earlier experiments the intradermal dosage of antigen was 0.1 c.c. It was found, however, that doses of 0.05 c.c. seemed to give equally satisfactory differential results, with less chance of producing lesions due to the technique of injection. It is possible that even smaller doses will suffice and that the reaction has a qualitative basis rather than a quantitative one, i.e., dependent more on the concentration of the antigen than upon the actual amount injected.

Some of our results suggested that an antigen which had been kept in the ice-chest for 8-12 days was better differentially than one which was but freshly prepared. Such old antigens seemed to have lost most of any primary toxicity for normal monkeys, while still retaining their potency in infected ones. This age factor may possibly be concerned with a further disintegration of the parasite proteins by continued slow papain digestion or autolysis in the ice-chest, which may result in a diminution of any non-specific reactive digestion products.

From the results of these observations it seemed as if alcoholic precipitation tended to fix these variable properties, in accordance with the age of the antigen at the time of precipitation. Further experimentation will, however, be needed to confirm these suggestions, as different monkeys show some variation in the degree of reactivity to the same antigens.

#### RESULTS OF TESTS IN NORMAL AND INFECTED MONKEYS.

As stated above, differences in the dosage and strength of the antigens were liable to cause variations in the reactions observed. The general descriptions given below are chiefly of those obtained with a dosage of 0.05 c.c. of antigens of which the strength had been standardized. The injections were made into the shaved skin of the anterior abdominal wall with a tuberculin syringe.\*

The descriptions given contrast in a general manner the effects seen in normal monkeys (*S. rhesus*), with those in monkeys suffering from acute and chronic infections with the strain of malaria parasite obtained from the School of Tropical Medicine, Calcutta.

A total of 35 tests was made of various papain digests of parasites and infected organs on 9 acutely infected and 4 chronically infected monkeys, while 44 control tests with these antigens were carried out on 9 normal animals. The appearances in normal, as contrasted with infected monkeys, are shown graphically in Plates I to III, while in Plate IV are given some untouched photographs

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\* The method described by Dunn (1932) for controlling monkeys during experimental work was found very convenient, both at the time of injection and while making observations upon the reactions.

of the reactions. Fuller details of the results are given in the protocols at the end of the paper. Control injections of normal saline solution produced no effects either in normal or in infected monkeys.

*Appearances in normal monkeys.*

Within a few minutes after the injection of the antigen, a distinctly raised, circumscribed, pale swelling about 8 mm. in diameter developed around the point of injection. Marked goose-skin quickly appeared over and around this area, which increased gradually in size, so that at the end of 15 minutes the reaction had the appearance of a large, clearly defined, pale swelling about 10–20 mm. in diameter. Goose-skin was very marked at this time and usually extended for some distance beyond the swelling. There was, however, seldom any redness, except in some instances when a very strong antigen had been used. Even in the latter case petechiæ were never present at this time in normal monkeys.

After 3–4 hours the swelling has subsided and the reaction has almost gone. In some instances the area still shows a slight yellowish pallor and more rarely a small, red, pin-head papule may be present at the point of injection.

After 24 hours it is usually difficult to find the site of injection, which at most may still show a small pink papule. After 48 hours there are commonly no signs of the injection.

This reaction in normal monkeys closely resembles the 'immediate reaction' which occurs in some allergic conditions, and which Coca (1928) has classified as 'atopic hypersensitiveness'. It is probably due to some of the products of parasite digestion, as no such appearances were seen with control injections of normal saline solutions or even with Witte's peptone (5 per cent).

*Appearances in monkeys suffering from acute infections.*

The appearances within a few minutes after injection usually show definite differences from those seen in normal monkeys. The swelling is much flatter and less markedly circumscribed. Goose-skin is usually absent and, if present, quickly disappears leaving the centre of the swelling with a smooth surface. The size of the swelling is less extensive than in the normal and may be pinkish rather than white in colour, especially towards the centre.

After 15 minutes the swelling remains smaller, flatter and less definitely circumscribed than in the normal, being usually about 10 mm. in diameter. Any goose-skin seen earlier has almost disappeared and redness over the centre of the swelling is not uncommon. At this stage in acute infections, when injections of 0.1 c.c. are given, it is not rare to see a commencing ring of small petechial hæmorrhages delimiting the swelling. Sometimes commencing cuticular necrosis may be observed near the point of injection.

After 3–4 hours the reaction has increased to become a pale yellowish or greenish-pink, slightly raised, button-like swelling about 10–25 mm. in diameter with a smooth surface. At the centre of this is a small area, usually about

5 mm. in diameter, which may show different degrees of intense reaction, varying from a red hyperæmia usually with distinct cuticular necrosis, even up to a purplish, petechial condition or a dark area of deeper necrosis or cutaneous hæmorrhage, especially when larger doses or stronger antigens are used. The periphery of this button-like area usually shows a distinct, but irregular, petechial areola of a width varying from 2 to 5 mms.

At the end of 24 hours the reaction is usually at its maximum and is much more intense than at the previous observations. There is a distinct swelling about 20–30 mms. in diameter, at the centre of which there is a definite area of necrosis, which varies in extent from a mere involvement of the superficial cuticle to that of the whole thickness of the skin. An increased width and intensity of the petechial areola seems to be a characteristic feature of this stage. The whole area of reaction is sometimes very cedematous and indurated.

After 48 hours the reaction usually shows little or no increase in severity. Often there are signs of commencing resolution and the necrotic area has become either a deep punched-out ulcer or a black, depressed and puckered eschar. Complete healing takes a long time, often some weeks in a severe case.

The severity of the delayed reaction seemed to show a relation to the acuteness of the infection, and to the number of parasites present in the peripheral blood at the time when the tests were made.

In three monkeys which died with acute infections from 3 to 12 hours after the injections, the post-mortem appearances of the reactions were interesting. It was found that the delayed reactions were accompanied by severe sub-cutaneous hæmorrhage and congestion, extending much beyond the area noted during life and of much greater severity than was expected from the superficial appearance of the skin.

#### *Appearances in monkeys suffering from chronic infections.*

The reaction after a few minutes often showed some goose-skin especially at the periphery, but this disappeared much more rapidly than in normal monkeys. A distinct delayed reaction of a similar character to that seen in acute infections occurred, but was usually of a milder type.

#### *Comparison of reactions.*

The 'delayed reaction' seen in acute and chronic malarial infections in monkeys appears to be an example of the condition which Coca (1928) has called 'hypersensitiveness of infection'. Like this reaction it does not seem to be passively transferable by injections of serum from an infected monkey into a normal one. The typical examples of such hypersensitiveness are the tuberculin, the mallein and the typhoidin reactions. The reaction here described closely resembles the tuberculin reaction in that it occurs in infected and not in normal animals, the reaction is more severe in acute infections than in chronic ones, whole micro-organisms do not give the reaction to produce which some disintegration is necessary, and the development and appearances of the local lesions strongly recall the effects of tuberculin in tuberculous animals.

Antigens prepared from the livers and spleens of infected monkeys showed similar results in the few tests carried out, but it seems as if their effects were more severe, both in the normal and the infected animal, than those obtained with pure parasite digests (*vide* Protocols). This may possibly be due to the necessary presence of large amounts of disintegration products of tissue protein from the organs digested.

COMPARISON OF INTRADERMAL REACTIONS IN NORMAL AND INFECTED MONKEYS.\*

Time.	Normal monkey.	Infected monkey.
Up to 3 mins.	Distinctly raised and very circumscribed, pale swelling, which quickly develops marked goose-skin, often extending over the surrounding area.	Swelling much less distinctly raised and circumscribed. Goose-skin usually absent in acute infections, but may be slight in chronic cases; if present it is seldom seen over the centre of the swelling. Size of swelling much smaller than in normal control and may be pink rather than whitish.
15 mins.	Large circumscribed and raised swelling about 10–20 mm. in diameter. Goose-skin marked, usually extending beyond swelling. Seldom any redness and no petechiæ.	Swelling smaller and less circumscribed than in normal. Goose-skin, if present, very scanty. Redness of centre of swelling not uncommon. Sometimes commencing petechiæ and cuticular necrosis.
3–4 hours.	Distinct fading of primary reaction. Goose-skin gone. Swelling markedly subsided and area of primary reaction may have returned, more or less, to its original colour, or perhaps shows only a slight yellowish pallor. More rarely the point of injection may have a pink, pin-point papule.	Marked increase in the degree and extent of reaction. Pale yellowish or greenish-pink, slightly raised, button-like area, 10–25 mm. in diameter with a smooth surface. At the centre of this is a small area about 5 mm. in diameter which may be deep pink and hyperæmic, purplish and petechial or even dark and necrotic, according to the severity of the reaction. The periphery of the button-like area has usually a distinct petechial areola of variable extent and intensity.
24 hours.	Usually only the mark of the needle or a small pink papule left.	Reaction is at its maximum, showing considerably greater intensity than at 3–4 hours. Definite swelling about 20 mm. in diameter with central necrosis or petechiæ and destruction of superficial cuticle. The petechial areola is usually wider and more intense, a condition which is a constant feature of this stage. Usually there is distinct œdema of the surrounding tissues and the whole swelling is indurated.
48 hours.	Usually no signs left.	Usually little change since last observation, or perhaps commencing resolution, which may take a long time, especially if necrosis has been marked.

\* *Vide* Plates I–IV.

## DISCUSSION OF RESULTS.

From the results recorded above it will be seen that two distinct reactions occur after the injection of the antigen—a mild immediate reaction in normal monkeys and a more severe delayed one in infected animals.

The immediate reaction obtained with normal monkeys may possibly be of an anaphylactoid nature, such as is seen after the injection of histamine and other protein derivatives, in which case it may be due to the formation of some body of this character during the process of papain digestion. Another possible explanation is that the reaction is due to the 'endotoxin' of the malarial parasite, which has more effect on normal monkeys than on those which have been partially desensitized to it, by the frequent previous stimulation which occurs at the time of rupture of the mature schizont into the blood. Such a desensitization towards the immediate effects of the inciting agent, as the result of repeated stimulation, is a distinct feature of immediate skin reactions in some other pathological conditions.

The delayed reaction, which occurs in acute and chronic infections, has very many points of resemblance to the conditions which Coca (1928) has classified as 'hypersensitiveness of infection' and of which the principal examples are those obtained with tuberculin, mallein, typhoidin, etc.

In the case of protozoa, Wagener (1923) has evolved a skin reaction of this type for leishmaniasis. In this procedure the antigen is obtained by using cultured parasites, which are allowed to autolyse or dissolve in Coca's alkaline solution for several days. It seems possible that the antigen produced may have a similar basis to the one described here, except that in the latter instance the disintegration of the parasites was facilitated by the enzyme, papain.

The principle of disintegration of the parasite or 'liberation of the endotoxin', seems common to all the methods for the preparation of the 'antigens' used in these delayed reactions. Protein derivatives of the pathogenic organism are obtained by methods which seem to depend largely upon autolysis or enzyme action, so that the methods used are along the same lines as those employed by us to obtain an 'antigen' from the malaria parasite.\*

In the case of the tubercle bacillus, the pneumococcus and some other bacteria, preliminary digestion or autolysis of the bacteria by various methods seems necessary before a reactive antigen can be produced (Zinsser and Grinnell, 1927). In a similar manner we were unable to obtain a delayed skin reaction when a freshly prepared suspension of malarial parasites was injected intradermally into infected monkeys, nor did the malarial pigment, left after prolonged digestion of the parasites, produce any perceptibly abnormal reaction. As a result of their work with pneumococci, Zinsser and Grinnell (1927) say 'it appears reasonable to suppose that substances analogous to

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\* Several workers have commented upon the difficulty of disintegrating bacteria so as to obtain reactive antigens for cutaneous reactions. The experiments recorded above suggest that possibly papain may be worthy of a trial for this purpose.



our pneumococcus autolysates may be liberated from the bacteria within the body in the case of many organisms.....' 'Such an assumption would readily explain the well-known differences between the so-called "infection" and "injection" responses, and suggest an explanation of the true nature of the tuberculin reaction'.

The delayed reaction which occurs does not seem to be of the nature of local anaphylaxis, the Arthus' phenomenon, for some attempts at passive sensitization of the skin of normal monkeys by intradermal injections of serum from an animal with acute malaria, has been unsuccessful.

Several uncompleted investigations, which are in progress, suggest that the site of the reaction is cellular and not humoral. There would seem to be a sensitization of the local cells at least, as the result of infection.

Various workers, who have used the delayed skin reaction in the diagnosis of other diseases, have found that there appears to be no correlation between the occurrence of this reaction and the presence of demonstrable humoral antibodies. The fact, that the presence of such antibodies in malarial infections is still a matter of considerable doubt, supports the view that the reaction described may be mainly intracellular rather than humoral.

It is well established that the reticulo-endothelial system is very closely related to the destruction of the malarial parasite in the body. Indeed, the work on avian malaria suggests that the chief defence against malarial infection is phagocytosis by the cells of this system (Taliaferro, 1930). It is obvious, from the post-mortem examination of the internal organs in malaria, that the cells of the reticulo-endothelial system take up and rapidly digest free malarial parasites, and one would not expect that such phagocytosis and digestion would be without effect on those cells which survive. It is known that the stimulation results in a hypertrophy of this system and it seems probable that these effects may lead, either directly or indirectly, to the sensitization which appears to be present.

It has been suggested that the delayed effect is due to a reaction between the antigen and an intracellular antibody in the reticulo-endothelial system. It is possible that this may occur and that reaction may be severe enough to produce local lesions, only when the antigen is present in very great amounts or concentration, as may occur after intradermal injection. This point requires elucidation.

Some of the observations made during this research suggest that although there may be some general hypersensitiveness of the reticulo-endothelial system to the products of the disintegration of other proteins, yet this is comparatively slight in degree and the major part of the reaction recorded is probably due to specific products derived from the plasmodia used.

Whether the sensitization is specific either for one or all species of the genus *Plasmodium*, or whether it is a general phenomenon which may appear in other diseases, such as kala-azar, where the reticulo-endothelial system is also largely involved, will need further investigation.

## SUMMARY.

1. A disintegration product of a malarial parasite found in monkeys has been prepared by papain digestion.

2. This digest causes an immediate skin reaction when injected intradermally into normal monkeys, and the reaction seems to be of the type associated with 'atopic hypersensitiveness' (Coca, 1928).

3. Intradermal injection into monkeys, infected with the same strain of parasite, gives rise to a delayed reaction similar to that seen in the 'hypersensitiveness of infection' (Coca, 1928).

4. While these reactions show marked differences in infected as compared with non-infected monkeys, there is as yet no proof that they are specific for malaria in general, or even for infection with the special strain of malarial parasite used.

5. Some findings suggest that the delayed reaction is due to a sensitization of the cells of the reticulo-endothelial system, locally at least, as the result of malarial infection.

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## PROTOCOLS.

The results of the intradermal reactions carried out with different antigens are given below in the briefest form. In the majority of instances readings were recorded almost immediately after injection and thereafter at intervals of approximately 15 minutes, 3 to 4 hours, 24 hours, 48 hours, and at some later period. It is not possible here to describe fully the appearances noted in every test at these intervals. The most striking differences between the reactions occurring in normal and infected monkeys were to be seen at two definite periods after injection, namely (a) within about 15 minutes of giving the injection ('immediate reaction'), and (b) about 24 hours after injection ('delayed reaction'). The various stages in the development of some of the reactions are shown graphically in Plates I and II, while in Plate III some typical examples of 'immediate' and 'delayed' reactions are figured.

In the brief summary of the tests given below reference has been made only to 'immediate' and 'delayed' reactions.

*I. Results obtained with antigens prepared from parasites isolated from the blood of monkey No. 35.*

The clear filtrate, obtained after filtration of parasites digested with papain by the method described above, was stored in the ice-chest and used for intradermal tests on the monkeys, at the times specified below

## ANTIGEN No. 10.

*Antigen No. 10. (a) Pure filtrate after storage \* for 7 days.*

The following monkeys were used for testing this antigen:—

Nos. 38 (normal), 32 (acute malaria) and 7 (chronic malaria). The dosage employed was 0.1 c.c.

*Normal. Immediate.*—Slightly raised pale area 12 mm. in diameter.

*Delayed.*—Slightly raised pale pink area 10 mm. in diameter, with some oedematous swelling extending towards the umbilicus.

*Acute. Immediate.*—Pale flat area 10 mm. in diameter.

*Delayed.*—Severe reaction; necrosis and ulceration at site of injection; raised oedematous area extending downwards with extensive petechial discoloration over it. (Plate IV, fig. 10, shows this reaction 6 hours after injection).

*Chronic. Immediate.*—Distinctly raised, pale area 10 mm. diameter with marked goose-skin at periphery.\*

*Delayed.*—Severe reaction; extensive oedematous swelling with some petechial discoloration; site of injection marked by red area with necrotic centre and petechial areola. (Plate IV, fig. 9, shows this reaction 6 hours after injection).

*(b) Antigen No. 10 after storage for 12 days.*

The monkeys tested with this antigen were Nos. 40 (normal), 36 (acute) and 25 (chronic). Dosage of antigen was 0.1 c.c.

*Normal. Immediate.*—Pale raised area 12 mm. in diameter with marked goose-skin.

*Delayed.*—Slightly raised pink area 7 mm. in diameter with small oedematous swelling extending towards umbilicus.

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\* In these protocols 'storage' means storage in the ice-chest.

*Acute. Immediate.*—No record.

*Delayed.*—Severe reaction; pink area 20 mm. in diameter with central necrosis and petechial areola; extensive oedematous swelling extending towards the umbilicus, the skin over which shows marked petechial discoloration.

*Chronic. Immediate.*—Pale raised area 10 mm. diameter with goose-skin marked especially at periphery.

*Delayed.*—Very severe reaction; necrotic area 20 mm. diameter with central ulceration; extensive oedematous swelling with petechial discoloration extending towards umbilicus.

(c) *Antigen No. 10 after storage for 14 days.*

Only one monkey was used for this test (No. 29 chronic). Dosage of antigen 0·1 c.c.

*Chronic. Immediate.*—Pale raised area 10 mm. diameter with goose-skin at periphery.

*Delayed.*—Slightly raised pink area 12 mm. diameter; extensive oedematous swelling with some petechial discoloration.

ANTIGEN No. 20.

*Antigen No. 20. Pure filtrate after storage for 12 days, and diluted with an equal volume normal saline.*

The monkeys used for testing this antigen were Nos. 40 (normal), 36 (acute) and 25 (chronic). Dosage of antigen 0·1 c.c.

*Normal. Immediate.*—Pale raised area 12 mm. diameter with marked goose-skin.

*Delayed.*—Pale slightly raised area 14 mm. diameter.

*Acute. Immediate.*—No record.

*Delayed.*—Slightly raised red area 10 mm. diameter, with petechial areola. (Plate IV, fig. 6).

*Chronic. Immediate.*—Pale raised area 10 mm. diameter with marked goose-skin.

*Delayed.*—Pale raised area 25 mm. diameter with central red area; petechial discoloration at periphery and also over oedematous swelling extending towards umbilicus.

ANTIGEN No. 21.

*Antigen No. 21. Pure filtrate after storage for 13 days precipitated in absolute alcohol (24 hours); redissolved in original volume.\**

Tests were carried out with this antigen on monkeys, Nos. 40 (normal) and 29 (chronic), using a dosage of 0·1 c.c.

*Normal. Immediate.*—Pale raised area 10 mm. diameter with slightly pink centre. (Plate I).

*Delayed.*—Very mild reaction; pale pink raised area 8 mm. diameter. (Plate I).

*Chronic. Immediate.*—Flat red area 14 mm. in diameter with commencing necrosis at centre. (Plate I).

*Delayed.*—Severe reaction; pale pink area 20 mm. diameter with central ulceration and well marked petechial areola. (Plate I; Plate IV, fig. 8, shows reaction after 3½ hours).

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\* In these protocols the time indicated in brackets after precipitation in alcohol means the period during which the precipitate was allowed to remain in the alcohol.

The fluid used to redissolve the precipitate was always normal saline solution.

## ANTIGEN No. 23.

*Antigen No. 23. Pure filtrate after storage for 15 days, precipitated in alcohol (48 hours); redissolved in original volume.*

This antigen was used in doses of 0·1 c.c. for tests carried out on monkeys Nos. 42 (normal), 40 (acute) and 38 (acute).

*Normal. Immediate.*—Raised area with marked goose-skin and pale pink centre. (Plate I).

*Delayed.*—Small slightly raised pale area. (Plate I).

*Acute. (No. 40). Immediate.*—Pale flat area with goose-skin at periphery. (Plate I).

*Delayed.*—Raised red swelling with central ulceration and extensive oedematous natural coloured swelling extending towards umbilicus. (Plate I).

*Acute. (No. 38). Immediate.*—Pale flat swelling with goose-skin at periphery. (Plate I).

*Delayed.*—Raised red area with central necrosis and some petechiæ at the margin. (Plate I).

## ANTIGEN No. 24.

*Antigen No. 24. Pure filtrate after storage for 15 days, precipitated in alcohol (10 days); redissolved in original volume.*

This antigen was used in doses of 0·1 c.c. for tests in monkeys Nos. 45 (normal), 46 (normal), 41 (acute) and 33 (acute).

*Normal. (No. 45). Immediate.*—Pale raised area with marked goose-skin.

*Delayed.*—Slightly raised pale pinkish area with pin-head central necrosis.

*Normal. (No. 46). Immediate.*—Pale raised area with marked goose-skin.

*Delayed.*—Flat pink area 10 mm. diameter with slight necrosis of cuticle at centre.

*Acute. (No. 41). Immediate.*—Pale pink flat area; no goose-skin.

*Delayed.*—Death occurred 12 hours after injection when the reaction was intensely severe, being characterized by a hæmorrhagic area about 30 mm. diameter with central necrosis and ulceration.

*Acute. (No. 33). Immediate.*—Small pale flat area; no goose-skin.

*Delayed.*—Death occurred about 3 hours after injection when the reaction was already intensely severe, being characterized by a hæmorrhagic area about 25 mm. diameter with a 10 mm. necrotic patch at the centre.

A single uncontrolled test was carried out on the previous day with this antigen (dose 0·1 c.c.) on monkey No. 9 (chronic).

*Chronic. Immediate.*—Small pale flat area with little goose-skin at periphery.

*Delayed.*—Slightly raised pale pink area 25 mm. diameter with 10 mm. necrotic area at the centre; some petechial discoloration at the periphery.

## Summary.

In every instance the results obtained with antigens prepared from parasites isolated from monkey No. 35 were very much more marked in the infected than in the normal monkeys. The presence of a very mild reaction in the normal monkeys suggested that the antigen was too concentrated. This was verified by diluting the antigen with an equal volume of normal saline for one series of tests (*vide* antigen No. 20), when a marked delayed reaction resulted in two infected monkeys while that in the normal control was negligible. Precipitation of the pure filtrate in absolute alcohol and re-solution of the soluble part of the precipitate in normal saline does not diminish, but rather enhances, the potency

of the antigen. When the precipitate was allowed to remain in alcohol for ten days before re-solution, the reactions resulting from its injection were more marked in both normal and infected monkeys than when antigen of the same age was allowed to remain in the alcohol for only 48 hours. As the stock of antigen was exhausted with the tests carried out, further experiments with more dilute preparations were impossible.

## *II. Results obtained with antigens prepared from parasites isolated from the blood of monkey No. 38.*

A papain digest of pure parasites was made in exactly the same way as from monkey No. 35. The pure filtrate was stored in the ice-chest and was used for the tests noted below.

### ANTIGEN No. 28.

#### *Antigen No. 28 (a). Pure filtrate after storage for 1 day.*

This antigen was used, in doses of 0.1 c.c. for tests performed on monkeys Nos. 45 (normal) and 41 (acute).

*Normal. Immediate.*—Pale raised area.

*Delayed.*—Small flat pale area 6 mm. diameter.

*Acute. Immediate.*—Small flat area 10 mm. diameter.

*Delayed.*—Death occurred about 12 hours after the injection was given. At this time the reaction was already intensely severe, being accompanied by central ulceration and marked petechial discoloration over an area about 20 mm. in diameter.

#### *Antigen No. 28 (b). Pure filtrate after storage for 6 days.*

This antigen was injected, in doses of 0.05 c.c., into two normal monkeys Nos. 48 and 49 during an attempt to standardize the strength and dosage of the antigens being used.

*Normal. (No. 49). Immediate.*—Raised pale area 10 mm. diameter with goose-skin.

*Delayed.*—Pin-head pink papule.

*Normal. (No. 48). Immediate.*—Raised pale area 10 mm. with goose-skin.

*Delayed.*—Slightly raised pale pink area 10 mm. diameter.

#### *Antigen No. 28 (c). Pure filtrate after storage for 11 days.*

A dose of 0.05 c.c. of this antigen was injected into each of monkeys Nos. 50 (normal) and 47 (acute).

*Normal. Immediate.*—Pale raised area 10 mm. diameter with marked goose-skin. (Plate II).

*Delayed.*—Nil. (Plate II; Plate IV, fig. 2).

*Acute. Immediate.*—Pale slightly raised area 7 mm. diameter; no goose-skin. (Plate II).

*Delayed.*—Raised button-like area with red centre and marked petechial areola. (Plate II; Plate IV, fig. 1).

#### *Antigen No. 28 (d). Pure filtrate after storage for 13 days.*

Injected into monkeys Nos. 51 (normal) and 39 (acute) in doses of 0.05 c.c.

*Normal. Immediate.*—Pale flat area 10 mm. in diameter with goose-skin.

*Delayed.*—Almost imperceptible pink spot. (Plate IV, fig. 4, shows this reaction 3 hours after injection).

*Acute. Immediate.*—Pale flat area 5 mm. in diameter with goose-skin.

*Delayed.*—Pink area 10 mm. in diameter with necrosis of cuticle at centre; marked petechial discoloration along inner border. (Plate IV, fig. 3, shows this reaction 3 hours after injection).

#### ANTIGEN No. 39.

*Antigen No. 39. Pure filtrate after storage for 2 days precipitated in absolute alcohol (24 hours); redissolved in original volume.*

(a) Preliminary tests were made with this antigen on two normal monkeys (Nos. 48 and 49) the dosage used being 0·005 c.c.

*Normal. (No. 49). Immediate.*—Area of marked goose-skin.

*Delayed.*—Flat slightly pale area about 10 mm. diameter.

*Normal. (No. 48). Immediate.*—Slightly raised pale area with marked goose-skin, especially at the periphery.

*Delayed.*—Flat pale pink area about 12 mm. in diameter.

(b) As these preliminary tests gave no appreciable delayed reaction in two normal monkeys, further tests were made on monkeys Nos. 50 (normal) and 47 (acute) five days later. Dosage 0·005 c.c.

*Normal. Immediate.*—Slightly raised pale area with goose-skin.

*Delayed.*—Small pink papule. (Plate IV, fig. 2).

*Acute. Immediate.*—Slightly raised pale pink area; no goose-skin.

*Delayed.*—Raised pink button-like area, with slight petechial discoloration at the periphery. (Plate IV, fig. 1).

(c) The same antigen was used in the same doses for further tests made two days later on monkeys Nos. 51 (normal) and 39 (acute).

*Normal. Immediate.*—Pale slightly raised area with goose-skin.

*Delayed.*—Nil. (Plate IV, fig. 4, shows this reaction 3 hours after injection).

*Acute. Immediate.*—Small flat pale area; no goose-skin. (Plate III).

*Delayed.*—Pink area 10 mm. in diameter, the skin over which is necrotic; small ulcerated area at centre and well marked petechial areola around. (Plate III; Plate IV, fig. 3, shows this reaction 3 hours after injection).

#### ANTIGEN No. 41.

*Antigen No. 41. Pure filtrate after storage for 2 days, precipitated in absolute alcohol (24 hours); redissolved in original volume.*

(a) Preliminary tests were made with this antigen on two normal monkeys (Nos. 48 and 49). The dosage employed was 0·005 c.c.

*Normal. (No. 49). Immediate.*—Raised area with marked goose-skin.

*Delayed.*—Nil.

*Normal. (No. 48). Immediate.*—Slightly raised area with marked goose-skin especially at the periphery.

*Delayed.*—Slightly raised pink area 8 mm. diameter.

(b) As these preliminary tests showed no marked reaction in normal monkeys, further tests were carried out five days later using this antigen in the same doses and of the same strength on one normal monkey (No. 50) and one infected monkey (No. 47).

*Normal. Immediate.*—Slightly raised area with marked goose-skin. (Plate III).

*Delayed.*—Nil. (Plate III; Plate IV, fig. 2).

*Acute. Immediate.*—Slightly raised pale area with a little goose-skin at periphery, which disappeared quickly. (Plate III).

*Delayed.*—Slightly raised pink area with some petechial discoloration at the lower border. (Plate III; Plate IV, fig. 1).

(c) Two days later two further tests were made on monkeys Nos. 51 (normal) and 39 (acute), using the same antigen in the same doses.

*Normal. Immediate.*—Slightly raised area with marked goose-skin.

*Delayed.*—Nil. (Plate IV, fig. 4, shows reaction after 3 hours).

*Acute. Immediate.*—Pale flat area; no goose-skin.

*Delayed.*—Flat pink area 10 mm. in diameter. (Plate IV, fig. 3, shows reaction after 3 hours).

#### ANTIGEN No. 45.

*Antigen No. 45. Pure filtrate after storage for 12 days, precipitated in absolute alcohol (24 hours); redissolved in original volume.*

This antigen was used for tests in monkeys Nos. 51 (normal) and 39 (acute) in doses of 0·05 c.c.

*Normal. Immediate.*—Flat pale area with marked goose-skin. (Plate III).

*Delayed.*—Almost imperceptible pink spot. (Plate III; Plate IV, fig. 4, shows this reaction after 3 hours).

*Acute. Immediate.*—Small flat pink area; no goose-skin. (Plate III).

*Delayed.*—Raised pale pink area with necrosis of skin at centre. (Plate III; Plate IV, fig. 3, shows reaction after 3 hours).

#### ANTIGEN No. 40.

*Antigen No. 40. Pure filtrate precipitated in absolute alcohol (24 hours); redissolved in double original volume.*

Preliminary tests were carried out with this antigen on two normal monkeys (Nos. 48 and 49). The results were similar to those obtained with antigen No. 39. No further tests were made with this antigen as antigen No. 36 proved to be of a more convenient strength.

#### Antigens Nos. 25, 26 and 27.

With the object of obtaining some information with regard to the optimum period of digestion with papain, which would yield the most potent antigen for intradermal reactions, samples were withdrawn at intervals during the process of digestion.

These samples were immediately precipitated in absolute alcohol and allowed to remain in it until the next morning, i.e., for a period of about 18 hours. The precipitate from each of these samples was redissolved in the original volume of normal saline and used for skin tests as follows:—

*Antigen No. 25. Sample (1) withdrawn after 105 minutes digestion.*

This antigen was used in doses of 0·1 c.c. for tests on monkeys Nos. 45 (normal) and 41 (acute).

*Normal. Immediate.*—Raised pale area with marked goose-skin.

*Delayed.*—Small raised pink area with pin-head central necrosis.

*Acute. Immediate.*—Slightly raised pale area; no goose-skin.

*Delayed.*—Death occurred about 12 hours after injection, when the reaction was already intense and characterized by a pale pink raised area 20 mm. diameter with well marked petechial areola and central ulceration.

*Antigen No. 26. Sample (2) withdrawn after 165 minutes digestion.*

Tests were made with this antigen on monkeys Nos. 45 (normal) and 41 (acute). The dosage was 0·1 c.c.

*Normal. Immediate.*—Raised pale area with marked goose-skin. (Plate III).

*Delayed.*—Nil. (Plate III).



*Acute. Immediate.*—Pale flat area with little goose-skin at periphery. (Plate III).

*Delayed.*—Death occurred after about 12 hours. At this time the reaction was well developed, being represented by a hæmorrhagic area 20 mm. in diameter with central necrosis of the skin. (Plate III).

*Antigen No. 27. Sample (3) withdrawn after 225 minutes digestion.*

Doses of 0·1 c.c. were injected into two monkeys, Nos. 45 (normal) and 41 (acute).

*Normal. Immediate.*—Raised pale area with marked goose-skin.

*Delayed.*—Small raised pink area with central linear scab.

*Acute. Immediate.*—Pale flat area with goose-skin at periphery.

*Delayed.*—Death of this monkey occurred about 12 hours after injection. At this time the reaction was characterized by a hæmorrhagic area about 20 mm. diameter, at the centre of which the skin was ulcerated.

### Summary.

Satisfactory results were obtained with all the antigens prepared from parasites isolated from monkey No. 38. Well marked differences were noted between the reactions in normal and infected monkeys in every instance. With better regulation of the concentration of the different antigens used, and the reduction of the dosage from 0·1 c.c. to 0·05 c.c. in the later tests, the results were, on the whole, more satisfactory.

### III. Results obtained with antigens prepared from parasites isolated from the blood of monkey No. 33.

The first digest, made from parasites isolated from this monkey, was made by exactly the same method as those prepared from monkeys Nos. 35 and 38. After filtration of the 4-hour digest the residue was diluted with a small quantity of distilled water, more papain was added and digestion was allowed to proceed for another four hours at 60°C. After filtration the filtrate from this 'redigest' was precipitated in alcohol; the precipitate was redissolved in normal saline after 24 hours, 2 c.c. saline being added for every 5 c.c. of the original filtrate.

During the process of dehæmoglobinization of the first batch, a number of parasites was left behind in the supernatant fluid at each wash. These parasites settled down as a deposit after being allowed to stand overnight, and from them a second batch of antigen was prepared by the same method as was employed for the first batch.

#### ANTIGEN No. 35.

*Antigen No. 35. Pure filtrate (first batch) after storage for 4 days.*

Preliminary tests were made with this antigen on two normal monkeys (Nos. 48 and 49). Dosage 0·05 c.c.

*Normal. (No. 49). Immediate.*—Pale raised area; marked goose-skin.

*Delayed.*—Pale flat area with some necrosis of skin at the centre.

*Normal. (No. 48). Immediate.*—Pale raised area with marked goose-skin.

*Delayed.*—Pale pink area with some central necrosis of skin and slight petechial discoloration at the periphery.

It was evident from these preliminary tests that this antigen was too concentrated. It was therefore diluted with an equal volume of normal saline before further use (*vide* antigen No. 43).

#### ANTIGEN No. 43.

*Antigen No. 43 (a). Pure filtrate (first batch) after storage for 9 days, diluted with an equal volume normal saline.*

Doses of 0.05 c.c. of this antigen were injected into monkeys Nos. 50 (normal) and 47 (acute).

*Normal. Immediate.*—Pale raised area with marked goose-skin. (Plate II).

*Delayed.*—Nil. (Plate II; Plate IV, fig. 2).

*Acute. Immediate.*—Pale raised area; no goose-skin. (Plate II).

*Delayed.*—Pale pink raised area with marked petechial areola. (Plate II; Plate IV, fig. 1).

*Antigen No. 43 (b). Pure filtrate (first batch) after storage for 11 days, diluted with an equal volume of normal saline solution.*

This antigen was injected into monkeys Nos. 51 (normal) and 39 (acute) in doses of 0.05 c.c.

*Normal. Immediate.*—Pale area with very marked goose-skin. (Plate III).

*Delayed.*—Nil. (Plate III; Plate IV, fig. 4, shows reaction after 3 hours).

*Acute. Immediate.*—Pale flat area; no goose-skin.

*Delayed.*—Flat pink area with red centre and petechial areola. (Plate III; Plate IV, fig. 3, shows reaction after 3 hours).

#### ANTIGEN No. 38.

*Antigen No. 38 (a). Pure filtrate (second batch) after storage for 2 days.*

Preliminary tests were made with this antigen on two normal monkeys (Nos. 48 and 49) using doses of 0.05 c.c.

*Normal. (No. 49). Immediate.*—Pale raised area with marked goose-skin.

*Delayed.*—Pin-head pink spot.

*Normal. (No. 48). Immediate.*—Pale raised area with marked goose-skin.

*Delayed.*—Raised pink area 10 mm. diameter.

As no appreciable delayed reaction occurred in either of these normal monkeys no further dilution was considered necessary.

*Antigen No. 38 (b). Pure filtrate (second batch) after storage for 7 days.*

Doses of 0.05 c.c. were injected into monkeys Nos. 50 (normal) and 47 (acute).

*Normal. Immediate.*—Raised pale area with marked goose-skin.

*Delayed.*—Small pink papule. (Plate IV, fig. 2).

*Acute. Immediate.*—Raised pale area with goose-skin at periphery.

*Delayed.*—Pink area 8 mm. diameter with central necrosis of skin and well marked petechial areola. (Plate IV, fig. 1).

*Antigen No. 38 (c). Pure filtrate (second batch) after storage for 9 days.*

Monkeys Nos. 51 (normal) and 39 (acute) were injected with 0.05 c.c. of this antigen respectively.

*Normal. Immediate.*—Raised pale area with marked goose-skin. (Plate II).

*Delayed.*—Small raised pink papule. (Plate II; Plate IV, fig. 4, shows reaction after 3 hours).

*Acute. Immediate.*—Flat pinkish area with some goose-skin at periphery. (Plate II).

*Delayed.*—Slightly raised pale pink area with central necrosis of skin; some oedematous swelling extending towards umbilicus. (Plate II; Plate IV, fig. 3, shows reaction after 3 hours).

#### ANTIGEN No. 37.

*Antigen No. 37 (a). Pure filtrate (redigest) after storage for 3 days, precipitated in alcohol (24 hours); redissolved in 2 c.c. for every original 5 c.c.*

Preliminary tests were made with this antigen on two normal monkeys (Nos. 48 and 49) using doses of 0.05 c.c.

*Normal. (No. 49). Immediate.*—Pale raised area with marked goose-skin at periphery.

*Delayed.*—Small raised pink papule.

*Normal. (No. 48). Immediate.*—Pale raised area with goose-skin at periphery.

*Delayed.*—Slightly raised pale pink area with few petechiæ along one margin.

The reactions in these normal monkeys were so mild that the antigen was used for further tests without dilution.

#### *Antigen No. 37 (b) prepared as in (a).*

Doses of 0.05 c.c. were injected into monkeys Nos. 50 (normal) and 47 (acute).

*Normal. Immediate.*—Pale raised area with marked goose-skin. (Plate II).

*Delayed.*—Pin-head pink papule. (Plate II; Plate IV, fig. 2).

*Acute. Immediate.*—Pale raised area with goose-skin at periphery. (Plate II).

*Delayed.*—Raised pink area with central necrosis of skin and wide petechial areola. (Plate II; Plate IV, fig. 1).

#### *Antigen No. 37 (c) prepared as in (a) and (b) above.*

Doses of 0.05 c.c. injected into monkeys Nos. 51 (normal) and 39 (acute).

*Normal. Immediate.*—Raised pale area with marked goose-skin.

*Delayed.*—Nil. (Plate IV, fig. 4, shows result after 3 hours).

*Acute. Immediate.*—Flat pale area with slightly pink centre.

*Delayed.*—Raised red area with necrotic centre and petechial areola. (Plate IV, fig. 3, shows reaction after 3 hours).

#### ANTIGEN No. 36.

*Antigen No. 36. Pure filtrate (first batch) precipitated in alcohol (24 hours) immediately after preparation; redissolved in original volume.*

Preliminary tests were carried out with this antigen on two normal monkeys Nos. 48 and 49. Dosage 0.05 c.c.

*Normal. (No. 49). Immediate.*—Raised pale area with goose-skin and little pinkness at centre.

*Delayed.*—Slightly raised pale area with necrosis at site of injection and a few petechiæ at margin.

*Normal. (No. 48). Immediate.*—Raised pale area with goose-skin and little pinkness at centre.

*Delayed.*—Flat pale pink area with necrosis at site of injection and few petechiæ at margin.

As reactions of considerable severity occurred in both these normal monkeys, this antigen was diluted with an equal volume of normal saline solution.

## ANTIGEN No. 44.

*Antigen No. 44. Prepared by diluting antigen No. 36 with an equal volume normal saline solution.*

Doses of 0·05 c.c. were injected into monkeys Nos. 50 (normal) and 47 (acute).

*Normal. Immediate.*—Raised area with marked goose-skin.

*Delayed.*—Nil. (Plate IV, fig. 2).

*Acute. Immediate.*—Pale flat area; no goose-skin.

*Delayed.*—Pale flat area with pink centre and slight petechial areola. (Plate IV, fig. 1).

*Summary.*

Well-defined differential results were obtained in every case with antigens prepared from parasites isolated from monkey No. 33. More attention was given to regulating the concentration of the antigens, with the result that the reactions were more uniform, and equally differential.

*IV. Results obtained with digests prepared from organs of infected monkeys.*

Encouraging results were obtained with papain digests of organs from heavily infected monkeys. After removal, the organs were ground up in a mortar with glass dust, and about double the volume of water added. After the addition of papain, digestion was allowed to take place in the water bath at 60°C. for four hours.

Only a few tests have been made with antigens prepared from organs. The results of these tests are given below.

## ANTIGEN No. 30.

*Antigen No. 30. Pure filtrate after digestion of liver of monkey No. 32, after storage for 13 days; precipitated in absolute alcohol (48 hours); redissolved in original volume.*

Doses of 0·1 c.c. were injected into monkeys Nos. 46 (normal) and 33 (acute).

*Normal. Immediate.*—Raised pale area with marked goose-skin.

*Delayed.*—Raised pale pink area with central necrosis.

*Acute. Immediate.*—Flat pale area; no goose-skin.

*Delayed.*—Death occurred about 12 hours after injection. At this time the reaction had already assumed intensely severe characters, being represented by a central necrotic area about 20 mm. diameter surrounded by a 10 mm. petechial areola.

## ANTIGEN No. 31.

*Antigen No. 31. Pure filtrate after digestion of spleen of monkey No. 35; stored for 21 days; precipitated in absolute alcohol (48 hours) and redissolved in original volume.*

This antigen was used in doses of 0·1 c.c. in monkeys Nos. 46 (normal) and 33 (acute).


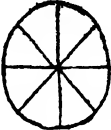
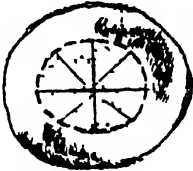
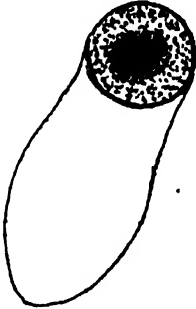






















*Normal. Immediate.*—Raised pale area with marked goose-skin.

*Delayed.*—Pale pink papule. (Plate III).

*Acute. Immediate.*—Flat pale area; no goose-skin.



























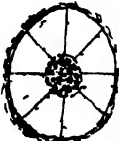





*Delayed.*—Severe reaction characterized by central hæmorrhagic patch, surrounded by pale pink areola with scattered petechiæ. (Plate III).

PLATE I.

Antigen No. Monkey No.	After 1-3 minutes.	After 15 minutes.	After 3 to 4 hours.	After 24 hours.
Antigen 23. Monkey 40. (acute).				
Antigen 23. Monkey 42. (normal).	No record.			
Antigen 23. Monkey 38. (acute).				
Antigen 21. Monkey 29. (chronic).				
Antigen 21. Monkey 40. (normal).	No record.			
 Raised area (a)	 Goose-skin (d)	 Blister or necrosis of cuticle (g)		
 Flat area (b)	 Pink or red area (e)			
 Pale area (c)	 Petechiae (f)	 Ulcer, scab, necrosis or haemorrhage (h)		

Stages in the Development of Intradermal Reactions in Infected and Normal Monkeys

PLATE II

Antigen No. Monkey No.	After 1-3 minutes.	After 15 minutes.	After 3 to 4 hours.	After 24 hours.
Antigen 28. Monkey 47. (acute).				
Antigen 28. Monkey 50. (normal).				
Antigen 37. Monkey 47. (acute).				
Antigen 37. Monkey 50. (normal).				
Antigen 38. Monkey 39. (acute).				
Antigen 38. Monkey 51. (normal).				
Antigen 43. Monkey 47. (acute).				
Antigen 43. Monkey 50. (normal).				

Stages in the Development of Intradermal Reactions in Infected and Normal Monkeys

PLATE III

Antigen Monkey	Immediate reaction	After 24 hours.	Antigen Monkey	Immediate reaction	After 24 hours.
Ant. 26 Monk.41		*	Ant. 38 Monk.47		
Ant. 26 Monk.45		,	Ant. 38 Monk.50		
Ant. 28 Monk.39			Ant. 39 Monk.39		
Ant. 28 Monk.51		.	Ant. 39 Monk.51		.
Ant. 31 Monk.33		**	Ant. 41 Monk.47		
			Ant. 41 Monk.50		.
Ant. 31 Monk.46		.	Ant. 43 Monk.39		
Ant. 37 Monk.39			Ant. 43 Monk.51		.
Ant. 37 Monk.51		.	Ant. 45 Monk.39		
* Death occurred about twelve hours after injection.			Ant. 45 Monk.51		.
** Death occurred about three hours after injection.					

'Immediate and 'Delayed' Intradermal Reactions in Infected and Normal Monkeys

#### EXPLANATION OF PLATE IV.

- Fig. 1. Intradermal reactions in monkey No. 47 (acute malaria), 24 hours after injection with antigens Nos. 43, 44, 37, 38, 28, 39 and 41.
- „ 2. Results of intradermal tests in monkey No. 50 (normal), 24 hours after injection of the same antigens as in Fig. 1.
- „ 3. Results of intradermal tests in monkey No. 39 (acute malaria), 3 hours after injection with antigens Nos. 43, 45, 37, 38, 28, 39 and 41.
- „ 4. Results of intradermal tests in monkey No. 51 (normal), 3 hours after injection with the same antigens as in Fig. 3.
- „ 5. Intradermal reaction in monkey No. 29 (chronic malaria), 3½ hours after injection with antigen No. 10.
- „ 6. Intradermal reaction in monkey No. 36 (acute malaria), 20 hours after injection with antigen No. 20.
- „ 7. Intradermal reaction in monkey No. 36 (acute malaria), 20 hours after injection with antigen No. 10.
- „ 8. Intradermal reaction in monkey No. 29 (chronic malaria), 3½ hours after injection with antigen No. 21.
- „ 9. Intradermal reaction in monkey No. 7 (chronic malaria), 6 hours after injection with antigen No. 10.
- „ 10. Intradermal reaction in monkey No. 32 (acute malaria), 6 hours after injection with antigen No. 10.



# PLATE IV

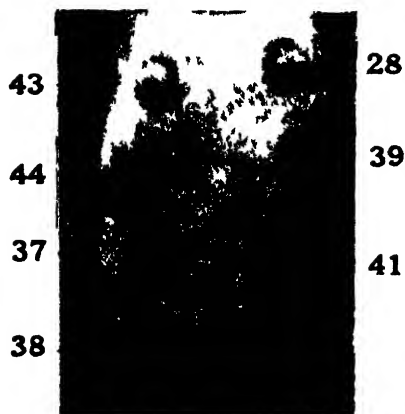


Fig. 1.

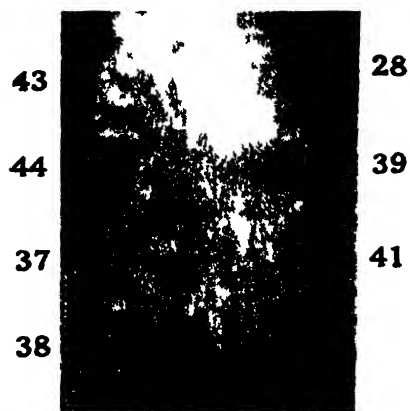


Fig. 2.

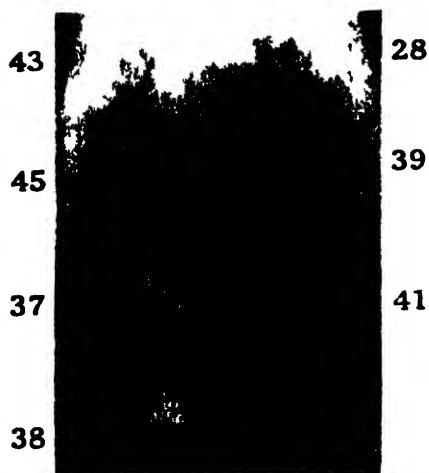


Fig. 3.

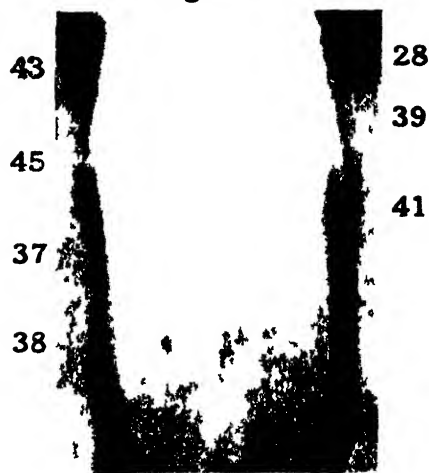


Fig. 4.



Fig. 5.



Fig. 6.



Fig. 7.

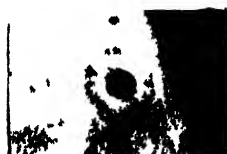


Fig. 8.



Fig. 9.



Fig. 10.



ANTIGEN No. 34.

*Antigen No. 34. Pure filtrate after digestion of liver of monkey No. 35, after storage for 21 days; precipitated in alcohol (48 hours); redissolved in original volume.*

This antigen was used in doses of 0.1 c.c. for tests in monkeys Nos. 46 (normal) and 33 (acute).

*Normal. Immediate.*—Pale raised area with marked goose-skin.

*Delayed.*—Slightly raised pale pink area.

*Acute. Immediate.*—Pale flat area; no goose-skin.

*Delayed.*—Death occurred after about 12 hours from the time of injection. At this time the reaction was very severe being characterized by extensive central necrosis and a marked petechial areola, the whole lesion being about 30 mm. diameter.

*Summary.*

Digests of heavily infected organs in every instance gave intensely severe reactions in infected monkeys. In one instance the reaction was negligible in the normal monkey (antigen No. 31), while in the two other cases a reaction of considerable severity occurred in the normal monkeys, although this was much less intense than that produced in the infected ones. It is probable that dilution of these antigens (antigens Nos. 30 and 34) might give very good results.



## HELMINTHIC INFECTIONS IN INDIAN ANOPHELINE MOSQUITOES.

BY

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(*Malaria Survey of India, Kasauli*).

[October 28, 1932.]

THE immature stages of both nematode and trematode worms have been reported in the adult and larval stages of Indian Anophelines, but these records are comparatively few, if one takes into account the number of mosquito dissections performed annually. As most of these records are widely scattered in literature, the principal ones are given here to aid other workers in India.

### (a) NEMATODES.

The presence of nematode larvæ in any stage of the development of the mosquito must always be of interest to workers in tropical medicine, since Manson's classical discovery of the transmission of human filaria by this insect. From the common occurrence of microfilaria in animals, especially in birds, in India, one would expect records of the presence of nematode larvæ in mosquitoes to be of much more frequent occurrence.

James (1900) described the metamorphosis of human microfilaria in *Anopheles subpictus* (rossi) and another Anopheline at Quilon, Travancore.

Chatterjee (1901) records 'five living filariæ' in *A. hyrcanus* in Calcutta. Perry (1912) reported that, in the Jeypore Hill Tracts of the Madras Presidency, two species of worm were found as parasites of adult Anophelines. At the commencement of the rains on some days 'a large filaria' was present in over 5 per cent of the Anophelines taken. This parasite was about  $\frac{3}{4}$  inch long and caused an irregular bulging of the abdomen of the host.

Carter, Rustomjee and Saravanamuttu (1927) found nematodes in over 100 Anopheline larvæ from 53 different breeding places in Ceylon. The larvæ affected were those of *A. aitkeni*, *barbirostris*, *culicifacies*, *hyrcanus*, *jamesi*, *listoni* and *subpictus*. The adults of *A. culicifacies* and *subpictus* were also found infected.

Iyengar (1929) in Bengal found worms belonging to the genus *Mermis*, family MERMITHIDÆ,\* in the larvæ of *A. varuna*, *ramsayi* (*pseudojamesi*), *hyrcanus* (*sinensis*), *barbirostris*, *fuliginosus*, *philippinensis* and *tessellatus*. These parasites were fairly common during the rains. They were from 5 to 8 mm. long and usually from 63 to 110 microns thick. He also found a larger, and what he considered to be a different, species of *Mermis*, in the adults of *A. fuliginosus* and *A. subpictus*. The latter worms were of two sizes—(a) 14–17 mm. long by 158–174 microns thick and (b) 8.3 mm. long and 138 microns thick. Iyengar (1929) studied the manner in which the immature nematodes attacked the larval stages of the mosquito. He gives descriptions of the morphology, growth and history of this parasite in the mosquito and while free-living.

Senior-White (1929) records the finding of a nematode in the larva of *A. barbirostris*.

Various workers have reported similar larvæ in Culicine mosquitoes in different parts of the world, but Howard *et al.* (1912) mention that Johnson found a nematode in the larva of an American Anopheline and Shakhov (1928) records these worms in the adults and larvæ of *A. maculipennis* at Kharkov in Russia.

Subedar J. D. Baily, I.M.D., of the Sind Malaria Enquiry, has sent me three larval nematodes obtained from a female specimen of *An. culicifacies* caught at Dodai, near Larkhana, Sind, on 3rd October, 1932. These worms were found entangled in the Malpighian tubes and were moving freely when dissected out.

The specimens were kindly examined for me by Captain V. T. Korke, F.R.C.P. (Edin.), who identified them as immature larvæ of *Mermis* sp. These specimens of *Agamomermis* were about 4.1 mm. long and 110 microns wide. There was a protruded spine about 100 microns long at the anterior end; the posterior end is bluntly rounded, and the body filled with granular material.

In a collection of Anopheline larvæ, which had been made from around Kasauli, Simla Hills, Punjab and mounted in Canada balsam for class demonstration purposes, four were found on later examination to be infected with nematode larvæ.

These larvæ were about 14 mm. long and were present as 3–5 long coils extending from the front of the thorax to the distal end of the abdomen of the insect larvæ. They seemed to fill the entire body. It was difficult to make

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\* MERMITHIDÆ are greatly elongated 'Nematodes', which, in the larval stage, are parasitic in insects, but in their adult condition are free-living. Cuticle with diagonal striation. Without open mouth or anus. Oral papillæ present. Characteristic eggs with two processes, ending in a tuft of filaments. Larvæ with a movable boring spine at the head end (Fantham, Stephens and Theobald, 1916).

The name *Agamomermis* Stiles, 1903, has been given to a collective group containing immature MERMITHIDÆ not developed to a stage which permits of a determination of the genus (Speer, 1927). Most of the larval nematodes found in mosquitoes have been placed provisionally in this group.

out any fine details of their structure, as these were obscured by the tissues of the larval host. The anterior end tapered for a distance of about 0.5 mm. to terminate in a bluntly rounded extremity about 50 microns wide. The remainder of the body had a uniform width of about 100 microns. The posterior end of the body showed no signs of tapering and terminated abruptly in a short wide transparent spine about 35 microns long.

From the anterior end, a very thin and wavy intestinal tract could be traced for a long distance, but no sign of this structure was visible at the anal end. The larva was surrounded by a thin transparent cuticle about 5 microns thick in which no signs of structure could be made out.

These nematodes were found in one larva of *An. gigas* var. *simlensis* and three of *An. lindesayi*.

Larval nematodes seem to have a markedly injurious effect on the insects which they parasitise. Perry (1912) and Carter *et al.* (1927) report that egg development appeared to be inhibited in the infected adult mosquito. The latter workers also state that the nematode found in the immature stages of Anophelines emerges during the pupal stage of the insect and seems to result in the death of its host. The parasites described by Iyengar (1929) apparently suppress the development of the imaginal organs of the infected larvæ and emerge from it before pupation occurs, killing the host instantaneously in the process.

#### (b) TREMATODES.

In Europe, Martirano (1901), Grassi (1901) and Schoo (1902) described encysted distomes in *Anopheles claviger*, while Ruge (1903) and Joyeux (1910) record one in *An. maculipennis*. Alessandrini (1909) considers that the parasites found in *An. claviger* are the larval forms of *Lecithodendrium ascidia* (v. Bendenen), a common parasite of the European bat (*Pipistrella europaei*). He thinks the bat eats the infected mosquitoes. Later when the faeces of the host fall into water, the eggs of the parasite hatch and the immature stages, after entering the mosquito larvæ, become encysted. Alessandrini (1909) is also of opinion that the parasites described by Martirano (1901) and Schoo (1902) are the same, but that of Ruge (1903) is a different species. The latter parasite he believes to be the larval form of *Distomum globiparum* described by Linstow from the snail, *Limnea ovata*, and of which the adult form is in the intestine of a fish.

The first record of a trematode in Anopheline mosquitoes in India seems to be that of Chatterjee (1901), who described and figured a specimen found in an adult *An. fuliginosus* in Calcutta. Stephens and Christophers (1902) also mention the finding of encysted bodies, like 'flukes', in the adults of *An. subpictus* (rossi) and *An. fuliginosus*.

Sinton (1917) described and figured encysted trematodes found in the adults of *An. listoni*, *culicifacies* and *stephensi* at Kohat, N. W. Frontier Province. He also recorded that some larvæ of *An. culicifacies* and *willmori*

were found infected. He thought that these might be the same as the *Agamodistomum*\* recorded in *An. claviger* (*Agamodistomum martiranoi* Stiles, 1903) or in *An. maculipennis* (*Ag. anophelis* v. Thiel, 1922) in Europe.

Soparkar (1918) remarked upon the resemblance between the encysted trematode reported by Sinton (1917) and similar encysted trematodes 'found on the fins of certain fresh-water fish as well as in the bodies of snails, chiefly *Planorbis exustus*' at Bombay. By placing infected snails in water with the larvæ of *An. subpictus* (*rossi*), he was able to find infection in 5 out of 13 adult mosquitoes which hatched out. With the larvæ of *Culex fatigans* only 3 out of 101 adults were infected under similar conditions. Soparkar (1918) thinks that the adult form of these trematodes develops in some larvicidal fish and that it closely resembles a *Clinostomum* of the family FASCIOLIDÆ.

Van Thiel (1922) found that 5 per cent of *An. maculipennis* taken at Leyden in Holland were infected with *Agamodistomum anophelis*. He gives a detailed description of this parasite and particular attention is drawn to the stylet which is often found in the encysted trematodes of Anopheline larvæ. This author (van Thiel, 1922, 1925), from a study of the European forms, has produced evidence that *Pneumonoeces variegatus* from the lungs of the frog, *Rana esculenta*, is probably the ultimate form of *Ag. anophelis* and of *Cercaria anophelis*, the latter occurring in the snail, *Planorbis vortex*. Eckstein (1922) also reported the cercariæ of distomes in the larvæ of *An. maculipennis* and thought their further development took place in the toad, *Bombinator igneus*, in the lungs of which he found a trematode like *Pn. variegatus*.

Van Thiel (1922), after a careful study of the subject, decided that the European species, *Ag. martiranoi* and *Ag. anophelis*, were distinct from the Indian one described by Sinton (1917) and Soparkar (1918), and proposed the name *Agamodistomum sintoni* for the Indian species.

In August 1932, a specimen of *Anopheles culicifacies*, which was collected at Pinjaur, near Kalka, Ambala District, was found infested with encysted trematode larvæ. The specimen was a female and about 60 cysts were found scattered through the body cavity, in both the thoracic and abdominal regions. These larvæ seemed to be identical with those described by Sinton (1917) from the same species of mosquito at Kohat, N. W. Frontier Province.

The infestation with larval trematodes is apparently acquired during the larval stage of the insect host. If one can judge from the number of adults found infected by Sinton (1917), it would not seem to have such a deadly effect on the developmental stages of its host as have the larvæ of nematode worms. Sinton (1917) recorded as many as 250 cysts in a single male of *An. listoni*, but, apart from the fact that the infestation was commoner in male than in female insects, noted no other special conditions.

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\* The name *Agamodistomum* Stossich, 1892, is used to designate an artificial group made to contain agamic distomes in which the characters are not sufficiently developed to permit of an exact generic determination (Speer, 1927).



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***ANOPHELES AITKENI* JAMES VAR. N. *PINJAURENSIS*.**

BY

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*Entomologist to the Malaria Survey of India.*

[November 3, 1932.]

*Anopheles aitkeni* James var. n. *pinjaurensis*. A small fragile brown Anopheline closely resembling the type form of *A. aitkeni*, but differing in the structure of the male hypopygium.

Female, and early stages, at present unknown.

**Male : Head :** Scales fairly numerous and moderately long, narrow, linear, and almost rod-like, dark in colour, some slightly expanded apically and bifurcate at tips, or ending in several irregular minute points; other scales on vertex narrow, hair-like, and with pointed ends. Eyes closely approximated above antennae, with a narrow space between bearing a few pale scales, and two pairs of fairly long hairs projecting forwards; one pair of latter on vertex at about level of upper margin of eyes, the other pair situated more anteriorly and arising from the narrow space between the eyes. Antennae plumose, the hairs brown, or light brown, according to the angle of light; shaft about  $\frac{3}{4}$ ths length of proboscis. Palpi slender, clubbed at extremities, brown, without pale markings, and slightly shorter than proboscis; the latter rather less than 2 mm. long, slender and entirely brown.

**Thorax :** Mesonotum brown, or yellowish-brown, according to angle of light. Scutellum and pleurae pale brown. Thorax apparently devoid of scales. **Wing :** Length 2.6 mm. Anterior forked cell almost  $\frac{1}{3}$ rd length of wing, and a little more than twice length of its petiole\*. Length of posterior branch of anterior forked cell rather more than twice the length of corresponding branch of posterior forked cell. Base of anterior forked cell much nearer base of wing than that of posterior forked cell. Scales on veins lanceolate; number of

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\* The petiole is that part of vein 2 between the cross-vein joining this vein to vein 3 and the bifurcation.



Fig. 1.—Male hypopygium of *A. aitkeni* var. n. *pinjaurensis* seen from dorsal, or tergal, side (coxite and style of one side omitted). Fig. 2.—Dorsal view of phallosome. Fig. 3.—Dorsal view of phallosome of *A. aitkeni*, type form, for comparison. Fig. 4.—Side view of phallosome of var. *pinjaurensis*. Fig. 5.—Side view of phallosome of *A. aitkeni*, type form, for comparison. Fig. 6.—Basal lobe and harpago of var. *pinjaurensis*. All figures drawn to same scale except Fig. 6, which is on a larger scale.

striations more usually 4 or 5. *Legs* : Long and slender, entirely brown, with a pale sheen when seen from certain angles.

*Abdomen* : Dark brown dorsally, paler ventrally, devoid of scales.

*Hypopygium* : The form of the phallosome was the most distinctive character found. This organ is very much longer than in *A. aitkeni*, type form, or in var. *bengalensis* Puri. Figures of the phallosome of var. *pinjaurensis* and of the type form are given for comparison. In dorsal view the apex of the phallosome around the opening is slightly expanded in the var. here described, but is without leaflets or other processes. In the closely allied *A. insulae-florum* Swellengrebel and Sw. de Graaf, there are spinous processes around the opening. Parabasal spines two, of about equal thickness, but with fine recurved tips, inner spine a little shorter than outer. A fairly well-developed spine near apex of coxite internally. Basal lobe and harpago very similar to those of var. *bengalensis*. The more dorsal lobe partly divided into two, one part with two sword-like spines (in *A. aitkeni*, type form, there are usually three spines in this position, but usually only two in var. *bengalensis*), the other part carrying two expanded, club-like, spines, apparently partly fused together. More ventrally there are two sword-like spines close together, and still further ventrally a single similar spine, and an accessory hair, much as in the type form and in var. *bengalensis*.

The type male of the var. No. Y. 1495 is in the Malaria Survey of India collection, Kasauli. It was caught at Pinjaur, Patiala State, near Kalka, Punjab, 19. iv. 1932 (*Jumna Dass* collr.). Although collecting has been going on in the same place regularly once a fortnight for a period of eighteen months only the type specimen has, so far, been obtained.



**A CRITICAL REVIEW OF THE LITERATURE RELATING TO  
THE IDENTIFICATION OF THE MALARIAL PARASITES  
RECORDED FROM MONKEYS OF THE FAMILIES  
CERCOPITHECIDÆ AND COLOBIDÆ.\***

BY

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**INTRODUCTION.**

THE recent researches which have been carried out on malarial infections in monkeys by workers in America, Malaya and India, have awakened a renewed interest in the malarial parasites of these animals.

Accurate identification of the parasite employed is one of the first essentials in carrying out any such experimental work. The importance of this is emphasized by a consideration of the clinical and therapeutic variations which have been observed in infections with the different species of the genus *Plasmodium* found in man.

The difficulties attendant upon the specific diagnosis of the Plasmodia of monkeys, were encountered by us in a study of the literature on this subject,

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\*Two families of the lower monkeys of the Old World are of special interest in connection with monkey malaria. These are the families COLOBIDÆ and CERCOPITHECIDÆ. The former family includes the langurs of Asia and the guerezas of Africa, while in the latter family occur the baboons of Africa and Arabia, the cercopithecques and mangabeys of Africa, and the macaques of Asia.

In the sub-family COLOBINÆ of the COLOBIDÆ, malarial parasites have been reported from two genera, *Colobus* and *Pygathrix* (*Semnopithecus*). In the sub-family CERCOPITHECINÆ of the CERCOPITHECIDÆ, the genera of interest in connection with the present article are *Papio* (*Cynocephalus*), *Cercopithecus*, *Cercocebus*, *Erythrocebus* and *Silenus* (*Macacus*).

The scientific nomenclature in relation to the Primates appears to be in a state of flux, and considerable differences of opinion seem to exist in the matter. In our work we have depended mainly upon the key catalogue published by Stiles and Nolan (1929), to which the reader is referred.

while attempting to identify or classify two strains of *Plasmodium*, which we had encountered as natural infections in two monkeys identified for us as *Silenus pileatus* (*Macacus pileatus*)\*. We considered it necessary to be able to differentiate these parasites with some degree of accuracy, before we could proceed with further researches into the specific nature of the intradermal test for malaria, with which we had been experimenting (Sinton and Mulligan, 1932, 1932a).

During our search through the literature, we were fortunate enough to be able to consult most of the papers on the subject in the original. As many of these articles are not easily obtainable by research workers in the tropics, we thought that the information which we have collected might be of use to such workers, and worthy of record.

It is not remarkable that the literature on monkey malaria should be in a confused state, when one considers the comparative paucity of the observations made and the difficult nature of the subject. The different species of human parasite were only defined after the examination of innumerable specimens from many hundreds of patients, and, in comparison with this, the work on the Plasmodia of monkeys has been infinitesimal. In spite of the enormous amount of work which has been done on the human parasite by workers all over the world, considerable doubt has existed as to whether there were more species of human *Plasmodium* than the three classically recognized. Only very recently one of the doubtful species, *P. ovale*, has been proved worthy of specific rank.

Many workers, who have made contributions to the literature on monkey malaria in recent years, have commented upon the difficulty in making accurate diagnoses of the parasites studied. Various authors, such as Bertarelli (1909), Blanchard and Langeron (1912), Leger and Bouilliez (1913), Wenyon (1926) and Leger (1928) have touched upon this problem, but, in spite of this, great confusion still exists. Inadequacy in the original descriptions has in some instances been responsible for this chaotic condition. Another factor, which in our opinion has contributed largely to the confusion, has been the tendency on the part of some workers to make their parasites fit in with the descriptions given by previous writers, and always to identify their parasites with recognized species, rather than to create new varieties or species. The result has been that, what may be called, 'collective species' have grown up.

Some authors are very reluctant to create a new species or variety, and it seems to us that this is the proper attitude to adopt, when few data are available. When, however, more evidence has been collected, it may eventually appear possible, reasonable and advisable, to subdivide into definite groups the miscellaneous parasites which have sometimes been gathered, for lack of sufficient knowledge, into a 'collective species'. Under such conditions we consider that the creation of definite named groups is essential to prevent further confusion of observations of all kinds, whether biological, morphological, pathological or developmental. These

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\* The proper name of this genus seems to be a matter of doubt and is given by various authors as *Silenus*, *Macacus*, *Macaca* and *Pithecus*.



groups may be called varieties of the parent species, if the evidence is considered to be sufficient. In this manner the end of scientific classification will be facilitated, i.e., means afforded for the more accurate identification of different species.\* It must, however, be recognized that eventually some of these varieties, or species may have to be sunk again in the parent species, when sufficient knowledge of individual variations has been collected, while others may be raised to, or may retain, specific rank. The grouping of parasites in the manner suggested is the first step towards a correct specific classification. A similar opinion has been expressed by Stiles and Nolan (1929).

At present there seem to be two main 'collective species' into which the Plasmodia of the CERCOPITHECINÆ are placed by most authors. The general rule for identification of the malarial parasites of these lower monkeys in the Old World seems to be that the Plasmodia of such Oriental monkeys should be called *Plasmodium inui*, while those of the African ones are grouped as *P. kochi*. We consider that there is now sufficient evidence for at least a tentative subdivision of these 'collective species' into several groups. These may for the present be called varieties of the type species.

We have attempted this task in the present paper and fully recognize that, when more complete and accurate evidence becomes available, very considerable modifications in our classification will almost certainly be required. The present confusion in the classification of monkey Plasmodia is great and is becoming worse yearly. Up to the present no one seems to have had either the opportunity, the patience, or the courage, to tackle this intricate problem in an exhaustive and critical manner. In the present paper we propose to consider only those Plasmodia which have been recorded as natural infections in monkeys of the families COLOBIDÆ and CERCOPITHECIDÆ (the lower Primates of the Old World). We hope that our investigation will be a step towards the elucidation of the problem or, at least, that the data collected here, may prove useful to other workers in the same field.

#### THE SPECIES OF *PLASMODIUM* RECORDED FROM THE MONKEYS OF THE OLD WORLD.

The following named species of *Plasmodium* have been reported from the blood of monkeys in the Old World :—

- (1) *P. (Laverania) reichenowi*† Sluiter, Swellengrebel and Ihle, 1922.
- (2) *P. kochi* (Laveran), 1899.
- (3) *P. bouilliezi* Leger, 1922.
- (4) *P. joyeuxi* Leger, 1928.
- (5) *P. cercopitheci* Theiler, 1930.
- (6) *P. pitheci* Halberstadter and Prowazek, 1907.

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\*It should always be remembered that scientific classification is not, as some authors would seem to think, an end in itself. The real object of such classification is rather a means towards the identification of a species, and to denote its relationship to other species.

†This is apparently the same parasite of the chimpanzee for which Blacklock and Adler (1924) proposed the same name—*P. reichenowi*.

(7) *P. inui* Halberstadter and Prowazek, 1907.

(8) *P. cynomolgi* Mayer, 1907.

(9) *P. semnopithecii* Knowles, 1919.

The first five species have been described from the blood of African monkeys and the last four from Oriental ones. *P. reichenowi* and *P. pitheci* were found in the higher apes, the chimpanzee and the orang-outang respectively. The natural hosts from which the other parasites have been recorded are some of the lower Primates, and it is with these parasites we propose to deal in the present paper.

While there is a certain similarity between most of the species of *Plasmodium* described from the lower monkeys, the majority seem to fall into two main divisions; those found in the African monkeys and those found in the Oriental ones. This appears to us to be a convenient manner in which to arrange these parasites until more precise data are available, and we have proceeded along these lines in the present paper.

This geographical division of the parasites is closely related to the two chief groups into which these Plasmodia can be divided on morphological grounds. Thus the majority of the Plasmodia recorded from the lower Primates of Africa can be included in the '*P. kochi* group' while most of those from the lower Oriental Primates belong to the '*P. inui* group'.

In our present state of knowledge, it is not easy to draw a hard and fast line between these two groups of parasite, each of which is composed of several varieties. In Table I we have contrasted some general characters, which we consider will indicate the main differences between the two groups. These characters will be dealt with in greater detail in the course of this paper.

TABLE I.

' <i>P. kochi</i> group'.	' <i>P. inui</i> group'.
(1) Natural infections occur in genera <i>Cercopithecus</i> and <i>Papio</i> .	(1) Natural infections occur in genera <i>Silenus</i> ( <i>Macacus</i> ) and <i>Cercocebus</i> .
(2) 'Accessory chromatin dot' never seen.	(2) 'Accessory chromatin dot' often present.
(3) Segmenting forms not seen in peripheral blood.	(3) All stages of parasite commonly seen in peripheral blood.
(4) No enlargement, pallor or stippling of infested red cells recorded.	(4) Enlargement, pallor and stippling of infested red cells often seen.
(5) Chromatin usually not abundant.	(5) Chromatin usually abundant.
(6) Pathogenic action usually slight.	(6) Pathogenic action variable; often severe.
(7) All attempts at transmission to other lower Primates have failed.	(7) Easily transmissible to other lower Primates.

## PLASMODIA FOUND IN THE BLOOD OF AFRICAN MONKEYS OF THE FAMILIES COLOBIDÆ AND CERCOPITHECIDÆ.

These families of monkey are represented in Africa by two sub-families namely the COLOBINÆ and the CERCOPITHECINÆ.

In the former sub-family, Plasmodia have only been recorded in one species, *Colobus rufimitratus*, from the Belgian Congo. These parasites were reported, without any description, by Strong and Shattuck (1930) and Theiler (1930).

The African monkeys of the sub-family CERCOPITHECINÆ include the following genera in which *Plasmodium* have been recorded:—*Cercopithecus*, *Cercocebus*, *Erythrocebus* and *Papio* (*Cynocephalus*)\*.

Koch (1898) was the first worker to record malarial parasites in monkeys. These, which he found in East Africa, were described by Kossel (1899) and named *Hæmamaeba kochi* by Laveran (1899). Under this name many other workers have recorded Plasmodia in African monkeys, but the chief criterion for these specific identifications seems, in many instances, to have been the finding of a malarial parasite in one of the African CERCOPITHECINÆ. This has resulted in considerable confusion and there is no doubt that under the name *P. kochi* (Lav.) many parasites of doubtful specific identity have been included.

Grall and Marchoux (1910) and Marchoux (1926) have also expressed the opinion that a more detailed study of such parasites may show that the '*P. kochi*' of different workers is composed of several distinct species of *Plasmodium*. Mesnil and Roubaud (1920) state that it has not been proved that *P. kochi* is the *only* parasite of African monkeys. Leger (1928) has taken up a similar attitude.

In addition to the malarial parasites of African monkeys which have been recorded as *P. kochi* (Lav.), several other species with considerable resemblance to this species have been reported. Leger (1922) described *P. bouilliezi* and also *P. joyeuxi* in 1928 (Leger, 1928). Macfie (1928) has reported and figured one, which he thought more closely resembled *P. inui*, the parasite described in Oriental monkeys, than *P. kochi*, the common African parasite. Theiler (1930) also found a monkey *Plasmodium* for which he proposes the name *P. cercopitheci*, if it should prove to be a new species.

The original description of *P. kochi* given by Kossel (1899) was very incomplete in the light of our present knowledge. However, Gonder and Berenberg-Gossler (1908) and Berenberg-Gossler (1909) gave detailed descriptions of a malarial parasite which they considered to be identical with Kossel's parasite. It has been on these later descriptions and on their coloured plates, that most workers seem to have based their identifications of *P. kochi* (Lav.), rather than upon Kossel's original work. From a close study of the description

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\* In Appendix I has been given a list of the various species of African monkey in the blood of which Plasmodia have been recorded. This list also shows the names of the different authors who reported these parasites, and their identification of the species of parasite found.

of *P. kochi* given by Kossel (1899) and of the descriptions and figures given by Gonder and Berenberg-Gossler, it seems to us that these workers were dealing with parasites of two different species (*vide infra*).

The two parasites described by Leger (1922, 1928) as *P. bouilliezi* and *P. joyeuxi* respectively seem to us to resemble more closely the original *P. kochi* of Kossel than does the *P. kochi* of Gonder and Berenberg-Gossler. There are, however, certain distinct differences between Leger's descriptions of his two parasites and Kossel's description of *P. kochi* (Lav.), but, in our present state of knowledge, it seems to us impossible to decide definitely whether or not these differences are real or only apparent. These may possibly be due to the incomplete description originally given by Kossel (1899), or to variations not yet recognized on account of the limited number of observations which have been made. We, therefore, propose that for the present Leger's two parasites should be known for the sake of convenience as *P. kochi* var. *bouilliezi* and *P. kochi* var. *joyeuxi*, until more reliable evidence is available of their specific identity. Such a procedure seems reasonable, more especially in view of the findings of Knowles and Das Gupta (1932), that the same species of monkey *Plasmodium* may show very distinct variations in morphology when inoculated into simian hosts of different genera.

### THE 'KOCHI GROUP' OF MONKEY PLASMODIA.

From a careful study of the literature on *P. kochi* and from a comparison of the descriptions of the different species of monkey *Plasmodia* reported from African monkeys, we propose that the parasites of this group be divided into four main divisions:—

- (a) *Plasmodium kochi* (Lav.) 1899, sens. restr.  
     'Malariaähnlichen Blutparasiten bei Affen'. Koch (1898); Kossel (1899).  
     *Hæmamaeba kochi*. Laveran (1899).  
     *Plasmodium kochi*. Sergent (1908).  
     *Plasmodium cercopitheci*. Theiler (1930).
- (b) *Plasmodium kochi* var. *bouilliezi* Leger, 1922.  
     *Plasmodium bouilliezi*. Leger (1922).  
     *Plasmodium kochi*. Anderson and Cowdry (1928).
- (c) *Plasmodium kochi* var. *joyeuxi* Leger, 1928.  
     *Plasmodium joyeuxi*. Leger (1928).  
     *Plasmodium* resembling *P. kochi*. Martoglio *et al.* (1910).  
     *Plasmodium kochi*. Joyeux (1913); Bouilliez (1916).
- (d) *Plasmodium kochi* var. *macfie* n. var.  
     *Plasmodium inui*. Macfie (1928).  
     *Plasmodium* sp. Seidelin and Connal (1914); Connal and Coghill (1916).  
     *Plasmodium kochi*. Theiler (1930).

(a) ***Plasmodium kochi* (Laveran) 1899, (sens. restr.).**  
 'Malariaähnlichen Blutparasiten bei Affen'. Koch (1898); Kossel (1899).  
*Hæmamaeba kochi*. Laveran (1899).

*Plasmodium kochi*. Sergent (1908).

*Plasmodium cercopithecii*. Theiler (1930).

Koch (1898) reported the discovery of parasites resembling human malaria parasites in the blood of several species of monkey in East Africa. Koch's material was examined by Kossel (1899), who described the parasite. To this parasite Laveran (1899) gave the name *Hæmamoeba kochi*.

Kossel (1899) studied material from a total of 62 monkeys. Of these 34 were brought by Koch from East Africa, 12 others were afterwards received from the same region, and 16, the origin of which was doubtful, were obtained in Berlin.

Of 27 *Cercopithecus sabæus* from East Africa, 15 were found to be infected, as was also one from an unknown source. Of 17 East African baboons,\* 3 showed parasites, and 4 out of 7 of unknown origin were also found infected. Among 7 monkeys of undetermined species, 3 had parasites in their peripheral blood.

Kossel (1899) illustrated his paper with 4 rather unsatisfactory coloured figures. His brief description may be summarized as follows:—

*In fresh preparations.* Scanty or numerous pale, globular bodies about the size of a red blood cell, possessing light yellowish brown pigment†; exflagellation observed.

*In preparations stained with borax-methylene-blue.* One part of the parasite stains a deep blue and granular, while the other part is pale, non-granular and greenish. Pigment appears dark in contrast with protoplasm. When the parasite occupies 1/3rd or more of infested cell, it may have an irregular shape, but is often ring-like with thickened sickle-shaped protoplasm at one side and round nucleus at other; sometimes no rings seen but elongated disc-shaped parasites with irregular margins. The variety of forms seen and observations on living parasites, suggested the conclusion that the parasite is amœboid at this stage. Pigment fine in all these forms, and increases with growth.

*In preparations stained with Romanowsky's methylene-blue and eosin mixture.* Young forms—signet-ring type; chromatin increases relatively less than protoplasm during growth, and is sometimes rounded, sometimes granular, sometimes in little curved rods. (In the 2 figures of asexual forms, the chromatin dot is very small and inclined to be slightly inside the vacuole.) In mature parasites the chromatin lies in an unstained vacuole. No dividing forms found.

Large forms (gametocytes) vary in staining reactions. One form‡ stains delicate green with methylene blue and has more chromatin; other form§ with less chromatin deep blue. Chromatin in first form a large mass of fine granules occupying about 1/3rd of parasite, while latter has coarser granules or rods. Latter forms more numerous. (Figures show uniformly distributed, very fine,

\* Martoglio *et al.* (1910), Leger and Bouillicz (1913) and Leger (1922, 1928) refer to these baboons as *Papio babuinus*, *Cynocephalus babuinus*.

† Apparently gametocytes.

‡ Apparently microgametocyte.

§ Macrogametocyte.

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yellowish brown pigment; nuclear background poorly stained but chromatin deeply; gametocytes free and about size of normal red cell.)

*Pathogenicity.* Neither Koch (1898) nor Kossel (1899) found any signs of illness in their infected animals. Young forms of parasite rarely seen in older monkeys. Failure to transmit by subcutaneous or intravenous injection of blood to other monkeys (? species).

The salient diagnostic characters of *P. kochi* (Lav.) would seem from Kossel's description to be the following:—*Asexual forms.* Small vacuolated rings about  $\frac{1}{3}$ rd diameter of red cell, with small amount of chromatin, which is sometimes rounded, sometimes granular and sometimes rod-like; protoplasm later becomes irregular and sometimes suggest amœboid activity; pigment appears early and in form of very fine, yellowish brown granules; older parasites elongated or rounded with irregular margins and small chromatin dot; no segmentation forms seen in peripheral blood; no change in size or staining of infested cell mentioned. *Sexual forms,* common; chromatin of nucleus small and stains deeply but rest of nucleus stains poorly; infested cell not enlarged (?). *Pathogenicity.* Slight, especially in old monkeys; inoculation experiments negative; natural hosts *Cercop. sabæus* and *Papio babunnus* (?) (*Cynocephalus babwinus*).

Sergent (1908) reported the occurrence of a malarial parasite in the blood of a specimen of *Cercopithecus albogularis* in the animal houses of the Pasteur Institute, Paris. He believes this parasite to be identical with *P. kochi* (Lav.) as described by Kossel (1899).

Sergent (1908) does not figure the parasite but gives the following description:—

*Sexual forms.* The only forms seen; size not greater than that of red cell; infested red cell showed no staining changes; pigment extremely fine and of ochre, not black, colour, uniformly distributed in both sexes of gametocyte. Nucleus stains with difficulty; on overstaining shows background of pale rose colour with deep red chromatin granules, isolated or in a mass; nucleus usually excentric, of variable size and larger in males than females; in former occupies about  $\frac{1}{3}$ rd parasite.

*Pathogenicity.* Monkey apparently in good health for 8 months; then, as result of injuries caused by daily capture and repeated handling, suddenly showed parasites in peripheral blood, with fall of rectal temperature and died in 5 days.

The type and colour of the pigment, the absence of asexual parasites and segmenting forms in the peripheral blood, the absence of any change in the infested cells, the size of the gametocytes, the poorly stained nucleus in the sexual parasites and the genus of the host (*Cercopithecus*), indicate that this parasite was probably *P. kochi* (Lav.).

Theiler (1930) records a *Plasmodium* from a specimen of *Cercopithecus nictitans* shot in Liberia. He proposes the name *P. cercopitheci* for this parasite, if it should prove to be a new species.

He gives a short description and 8 coloured figures\* of the parasite. The characters of this *Plasmodium*, as judged from his description and figures, would seem to be as follows :—

*Asexual cycle.* Heavy infection of blood; mostly rings of various sizes, showing one and fairly frequently two, almost equal, chromatin dots, but more often rod-form or sometimes U-shaped; pigment bright yellow and very fine, usually evenly distributed, but sometimes collected into one or two areas; infested cell not enlarged and no stippling seen. [His figures show (a) solid-looking ring about  $\frac{1}{3}$ rd diameter of infested cell, with two equal-sized, closely approximated, chromatin dots and no pigment; (b) a signet-ring form about  $\frac{1}{2}$  diameter red cell, with very fine protoplasmic ring, very small chromatin dot and no pigment; (c) an oval ring about  $\frac{1}{2}$  diameter of infested cell, protoplasm thickened at one side, two very small, equal chromatin dots at opposite diameters of, and slightly inside, large vacuole; (d) a somewhat similar parasite with two very small, equal, chromatin dots close together and slightly inside large vacuole; (e) larger parasite with large vacuole and blunt pseudopodium at one side containing pigment; the chromatin has two small, equal approximated dots on edge of vacuole and (f) an amœboid parasite with a blunt thick pseudopodium and large vacuole; chromatin U-shaped and relatively small; pigment fine and yellowish brown.]

*Sexual cycle.* Gametocytes completely fill the red cell; female stains deeply with dense compact nucleus; male stains less deeply, often appears yellow because of much finely divided pigment, nucleus large and diffuse. (Figures show two gametocytes apparently free, neither of these is larger than a normal red cell, they are filled with fine yellowish brown pigment. The karyosomes, but not the remainder of the nuclei, stain deeply and are relatively very small in both sexes.)

This parasite resembles *P. kochi* (Lav.) in the type, colour and comparatively early appearance of its pigment, the absence of change in the host cell, the absence of segmenting forms in the peripheral blood, the relatively very small size and the morphology of the nuclear chromatin at all stages, the amœboid activity of the trophozoites, the size of the gametocyte and the poor staining of the body of the nucleus in sexual forms.† From the close resemblance of this parasite to *P. kochi* (Lav.) as described by Kossel (1899), it does not appear to us that there are sufficient grounds for the creation of a new species, *P. cercopitheri*, or even of a separate variety of the former species.

From the above data it appears to us that until further and more detailed evidence is available, the name *P. kochi* (Lav.) in its restricted sense should be confined to a parasite having the following characteristics :—

*Asexual cycle.*—Young forms appear as circular or oval parasites, sometimes with only very small vacuole, but rapidly develop into typical ring forms, about  $\frac{1}{3}$ rd diameter of infested cell. Chromatin is comparatively small, rounded, rod-like or dumbbell-shaped. Older forms show some amœboid

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\* From preparations stained Giemsa's stain.

† The poor staining properties of the nucleus of the sexual parasites in *P. kochi* and its varieties, which have been mentioned and figured by various workers, appears to be due to the large amount of achromatic substance in the nucleus and the relatively small and compact chromatin mass (karyosome).

activity; very fine pigment of yellowish brown colour appears comparatively early; segmenting forms are absent from the peripheral blood (or possibly very rare). No enlargement or stippling of infested red cell present.

*Sexual cycle.*—Gametocytes are usually the commonest, and often the only, forms seen in peripheral blood. The nucleus is diffuse, stains poorly and has relatively small, compact karyosome; mature forms are little, if at all, larger than a normal red cell, and the infested cell shows no change of staining reaction.

*Pathogenicity.*—It has been recorded from the genus *Cercopithecus* and possibly from the genus *Papio* (*Cynocephalus*). The parasites usually seem to have little effect on the host, especially in older or chronically infected animals. It seems difficult to transmit the parasite to other monkeys by blood inoculation. \

**(b) *Plasmodium kochi* var. *bouilliezi* Leger, 1922.**

*Plasmodium bouilliezi.* Leger (1922).

*Plasmodium kochi.* Anderson and Cowdry (1928); Cowdry and Cowell (1928).

Leger (1922) recorded a *Plasmodium* from the blood of a freshly killed specimen of *Cercopithecus campbelli* in French Guinea. He considered this parasite to be a new species for which he proposed the name *Plasmodium bouilliezi*.

The following details have been obtained from the description and figures given by this worker.

*Asexual cycle.\** Trophozoites very rare in peripheral blood; youngest forms *not* rings, but elongated oval with one rounded and one pointed end, in which the karyosome lies in a bright blue protoplasm; no vesicular nucleus (figure shows a minute solid parasite about 1/8th the diameter of red cell with a very small chromatin dot). At later stage parasite appears stumpy, often triangular, with no tendency to amoeboid activity (figure shows parasite about 1/8th diameter of red cell, with very small chromatin dot and no vacuole). When parasite gets older and measures about 2.5–3 microns, protoplasm still remains compact; pigmentation black, in form of a few, relatively large granules (figure shows an irregularly oval, compact parasite with a small dumbbell-shaped nucleus). No segmenting-forms found in peripheral blood. No enlargement or other change detected in infested red cell.

*Sexual cycle.* Gametocytes fairly numerous in peripheral blood; usually free, rarely intracellular. Female usually oval, 7.5–8.5 microns, and therefore must cause certain mechanical enlargement of red cell before it becomes free; nucleus vesicular, unstained, and measures about 2.5 microns, with compact karyosome, rounded, oval or rod-like and very small; protoplasm without vacuole and packed with olive green pigment, uniformly distributed in large granules or clumps (figure shows very large, profuse, pigment granules in parasite which is larger than normal red cell). Male gametocyte slightly smaller than female, often deformed; nucleus usually diffuse and occupying about 1/2 parasite, with larger and deeply staining chromatin granules at one pole or in centre; pigment as in female, but much less clumped

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\* Specimens stained with Giemsa's and Leishman's stains.



and of a different golden yellow tint (figure shows numerous, very large pigment granules in parasite as large or slightly larger than normal red cell; chromatin scanty).

Leger (1922) distinguishes his parasites from the *P. kochi* of Gonder and Berenberg-Gossler (1908), of Luhe (1906) and of Bouilliez (1916) on the following points :—

Young schizonts not annular but compact; no distinct vesicular nucleus; growing schizonts non-amœboid and pigmented (Luhe and Bouilliez recognize fairly active amœboid movements in *P. kochi* and the latter describes no pigment);\* the gametocytes cause enlargement of the infested cell; pigment is in large grains or masses; its nucleus stains well, while Sergent (1908)† has emphasized the fine pigment and the poor staining character of the nucleus in the sexual forms.

Wenyon (1926) does not think there is sufficient evidence to distinguish this parasite from *P. kochi* (Lav.). Leger (1928), however, again records this parasite in a specimen of *Cercop. campbelli* from Guinea or Senegal.

Anderson and Cowdry (1928) figure the gametocytes of a *Plasmodium* found in two monkeys imported from Senegal, which they describe as 'genus *Callithrix* (closely resembling *C. personata*)'.‡ Apparently these workers only found gametocytes, and they identified their parasite as *P. kochi* (Lav.). They give no detailed description of the parasites but, from the coloured illustrations of Giemsa-stained preparations, the parasites would appear to have the following characteristics :—

*Sexual cycle.* Gametocytes free in plasma. Female larger than normal red cell and with faintly stained vesicular nucleus, having a round or rod-shaped chromatin dot, which is very small; pigmentation in numerous, very large granules of olive green or greenish blue colour, uniformly distributed through protoplasm. Male gametocytes said to be usually slightly smaller than female, but in figures also larger than normal red cell; nucleus diffuse and poor staining; numerous, very large, evenly distributed yellowish or light brown pigment granules. A very detailed description of exflagellation is given.§

*Pathogenicity.* Two monkeys were found naturally infected; infection chronic; passaged by blood inoculation to two other monkeys of same species, in which mild infections produced, tending to die out. One inoculated monkey showed a temperature of 39°C. with slight daily variations. Three monkeys died naturally and one was killed.

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\* *P. kochi* of Bouilliez (1916) would appear to be *P. kochi* var. *joyeuxi* Leger (1928) (*vide infra*) and probably not *P. kochi* (Lav.) sens. restr., so these pigment characters are not suitable for any differentiation from the type species, as described by Kossel (1899). As mentioned previously, *P. kochi* of Luhe (1906) from the chimpanzee is almost certainly not *P. kochi* (Lav.).

† This is probably true *P. kochi* (Lav.) (*vide supra*).

‡ The genus *Callithrix* are American monkeys belonging to the family CEBIDÆ and are not indigenous in Africa. If the diagnosis of the species of monkey be correct, this is the first record of the natural occurrence of *P. kochi*, an African species of parasite, in an American monkey. The description given by Anderson and Cowdry (1928) of the parasite seen by them appears different from any of the *Plasmodia* described from America.

§ Cowdry and Cowell (1928) carried out researches into the relative numbers of male and female gametocytes present in infections with this parasite.

The descriptions of the gametocytes of *P. bouilliezi* given by Leger (1922) and his figures, bear a very close resemblance to the parasite figured by Anderson and Cowdry (1928) and in our opinion the two appear to be identical. The natural hosts in both cases were obtained from West Africa, but the genera of monkey seem to be different in the two instances.

*P. kochi* var. *bouilliezi* Leger (1928) appears to differ from *P. kochi* (Lav.) mainly in the following points:—young schizonts more compact, and without vacuole; absence of amoeboid movement; gametocytes larger than normal red cell; pigment in very large, coarse grains and darker in colour (*vide* Table II).

### (c) *Plasmodium kochi* var. *joyeuxi* Leger, 1928.

*Plasmodium joyeuxi*. Leger (1928).

*Plasmodium* resembling *P. kochi*. Martoglio, Stella and Carpano (1910).

*Plasmodium kochi*. Joyeux (1913); Bouilliez (1916).

Leger (1928) reported and described a *Plasmodium* found in the blood of two specimens of *Cercopithecus callitrichus* from French Guinea or Senegal. Daily blood slides were made for nearly 8 months from one of these monkeys and Leger believes the parasite to be a previously unnamed species of monkey *Plasmodium*. He did not figure the parasite but gave the following description and named it *P. joyeuxi*:—

*Asexual cycle* Smallest rings 1·5–2·0 microns; annular, but not the classical signet-ring forms; chromatin *not* in a rounded mass, but elongated sausage-shape on two-thirds of periphery of parasite and surrounding the vacuole. Parasite usually in centre of red cell; double infections rare. Parasites soon become amoeboid and show surprising activity, producing extremely fine pseudopodia like *P. tenue*.

Older trophozoites never compact, protoplasm always sky-blue, obscured from place to place by very fine dust-like darkish ('noirâtre') pigment. Chromatin not forming compact homogeneous mass, but with bizarre aspect—irregular broken rod penetrating the pseudopodia, or filament in graceful spirals, or irregular dumbbell-shapes, or string of nodes, or broken ring with dilated ends. Only in more advanced stages is protoplasm condensed and does chromatin show tendency to clump; protoplasm may show deep clefts.

Dividing forms very rare; forms with 2 or 3 chromatin masses exceptionally seen, never mature segmenting forms; thinks these occur in internal organs as with *P. falciparum*, although not found on spleen or liver puncture.

Infested red cells not enlarged, except possibly in case of parasitized macrocyte; never any stippling of infested cell, though same technique showed with *P. vivax* and *P. falciparum*. Asexual forms occur less frequently and have shorter duration in peripheral blood than have sexual.

*Sexual cycle*. Gametocytes often in plasma, size slightly greater than normal red cell; females more numerous than males. Macrogametocytes with relatively well-developed nucleus, of compact mass of large chromatin granules; very marked vacuole; protoplasm with numerous, coarse pigment granules, not black but yellow-brown, sometimes greenish due to reflection from deep blue of protoplasm.

Microgametocytes with poorly delimited nuclear chromatin; no apparent vacuole; pigment lighter than in female, of ochre yellow or golden colour; cytoplasm violet. Exflagellation observed under proper conditions of temperature and humidity.

*Pathogenicity.* Slight fever in infected monkey when parasites present in peripheral blood; disease does not appear to affect monkey much. Attempts at transmission by subcutaneous inoculation to *Erythrocebus* (*Cercopithecus*) *patas*, *Cercopithecus callitrichus* and *Papio* (*Cynocephalus*) sp. always failed.

Leger (1928) distinguishes this parasite from *P. kochi* (Lav.), as described by Kossel (1899) and by Sergeant (1908), by the more deeply staining chromatin of the macrogametocytes, in which the pigmentation is very abundant and consists of large irregular granules of a brownish colour. Asexual forms may be numerous in the peripheral blood but no segmentation seen. Trophozoites have a very amœboid stage and the pigment in them is like black dust.

Joyeux (1913) reported the presence of a *Plasmodium* in 3 out of 9 specimens of *Cercop. callitrichus* in French Guinea. The forms seen were mostly gametocytes but a few rings were also observed. He attempted to transmit the infection to three other monkeys of the same species with negative results. This worker, from a comparison of his parasite with the figures of Berenberg-Gossler (1909), thought he was dealing with the same parasite, but Leger (1928) considers that this parasite is the same as *P. joyeuxi* Leger, 1928.

Martoglio, Stella and Carpano (1910) found a malarial parasite in *Cercop. sabæus* in Ethiopia. They give a plate of coloured figures\* and the following details are taken from their description:—

*Asexual cycle.* Youngest forms show a delicate granule of chromatin with very little protoplasm; many with chromatin set in very fine and small protoplasmic ring. (Figures show chromatin relatively very large, often U-shaped and occupying most of parasite.) Later ring thickens to oval form, sometimes with 2 equal chromatin dots united or separate; may get multiple infection of same cell. No change in size or staining of infested cell. As parasites grow, chromatin increases and becomes rod-like, sometimes with 2 or 3 nodules. (Figures show parasite with relatively very large amount of chromatin, situated on margin of parasite and sometimes U-shaped.)

When parasite about 1/4th to 1/3rd diameter red cell, it shows irregularly rounded form, often with pseudopodia; protoplasmic ring may be thickened on one side. No pigment at this stage; great development of chromatin and tendency for it to become large ring or solid peripheral ribbon; may see 2 or 3 chromatin masses united by filament, or forms simulating division. Infested cell of normal size; no staining changes recorded or figured. (Figures show some parasites with marked amœboid forms and very large amount chromatin of pleomorphic character, which may simulate early segmentation.)

When this form (rich in active chromatin, non-pigmented, very amœboid and with large vacuole) becomes about 1/3rd diameter of red cell, pseudopodia diminish and disappear. Protoplasm becomes deep blue; chromatin as granule, rod or small ribbon, proportionately less in amount than in previous form and

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\* From smears stained with Giemsa's and Marino's stains.

apparently inactive. Pigmentation begins, which described in fresh as yellowish mahogany. (Figures show this as very fine and dark brown.)

Parasites increase in size; their forms become regularly rounded, and, finally, after filling red cell become free in plasma; pigment more marked and granules larger.\* Complete segmenting forms never seen in peripheral blood.

*Sexual cycle.* Gametocytes usually free in plasma when full grown (*vide supra*). (Figures show mature forms as slightly larger than normal red cell.) Macrogametocytes with vesicular nucleus, poor in chromatin, which near periphery and consisting of 2 to 4 dark granules, usually related to unstained vacuole dividing nucleus from protoplasm; latter dark blue. (Figures show abundant dark brown pigment in fine granules, and nucleus devoid of staining, except for chromatin of karyosome.) Microgametocytes (figures show poorly stained parasite filled with fine golden brown pigment and large nucleus with diffuse chromatin of rose colour). Exflagellation described.

*Pathogenicity.* Marked anaemia in monkey; fever with tendency to tertian periodicity. Inoculation into *Silenus* (*Macacus*) negative.

Martoglio *et al.* (1910) emphasize the following characteristic features in their parasite :—resemblance to *P. falciparum* (precocious division of chromatin, slow amoeboid movement; long duration of apigmented stage; schizogony in internal organs; may find only gametocytes in peripheral blood); pathogenic action in *Cercopithecus* but not inoculable into *Silenus* (*Macacus*).

It will be seen that this parasite bears a close resemblance to the original description of *P. joyeuxi* Leger, 1928, in :—the absence of young signet-ring forms; irregular shape and large size of chromatin; occurrence of 'tenue' forms; long duration of apigmented stage of schizonts; absence of stippling or staining changes in cells infested with asexual forms; absence of schizogony in peripheral blood; common occurrence of gametocytes, which slightly larger than normal red cell and with abundant pigment of somewhat similar colour. Both occur naturally in *Cercopithecus* sp. with slight fever in infected host; attempts to inoculate to other monkeys unsuccessful. In our opinion these two parasites are the same.

Bouilliez (1916) found a *Plasmodium* in a specimen of *Cercopithecus callitrichus* at Moyen Chari, Central Africa. During the 3 weeks in which this monkey was under observation it showed gametocytes, and only for 6 days were asexual forms observed.

He figures some asexual forms and gives the following account of the parasite, which he calls *P. kochi* :—

*Asexual cycle.* Parasites sometimes amoeboid, sometimes regular oval or rounded; karyosome band-like along border of vacuole; sometimes double infection of cells. No enlargement of infected cells; no pigment seen in any asexual forms. [Figures show forms with large amounts of chromatin and large vacuole, closely resembling the figures of Martoglio *et al.* (1910). These schizonts are noted in the explanation of figures as having slight pigmentation, which is shown as fine granules.] Only two forms seen which suggested segmenting forms, one with 4 and the other with 7 chromatin masses and no pigment. [One of these forms has a close resemblance to figure 8 of Martoglio *et al.* (1910), showing a form which has no pigment and which these authors think is a trophozoite with irregular chromatin.]

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\* These presumably are gametocytes.

*Sexual cycle.* (Not figured.) Gametocytes with golden yellow pigment in fairly large ('assez gros') grains in the males and finer in the females. (This resembles the figures of Martoglio *et al.*, 1910.) When stained with Giemsa's stain, pigment in former turns blackish and in latter blue or green. Chromatin always surrounded by a clear area. Infested cells appear normal size. Exflagellation noted.

*Pathogenicity.* Inoculation into three *Cercopithecus callitrichus* and one *Erythrocebus patas* (*Cercopithecus patas*) gave negative results.

There is a very marked resemblance between the figures given by Bouilliez (1916) and some of those of Martoglio *et al.* (1910). The descriptions given by these workers also shows a close similarity with Bouilliez's parasite. In our opinion these workers were dealing with the same parasite.

*Plasmodium kochi* var. *joyeuxi* Leger, 1928, would therefore appear to differ from *P. kochi* (Lav.) sens. restr. in the following characters:—young forms not typical signet-rings; chromatin very large and of very irregular shapes; very amœboid 'tenué', asexual forms seen; pigment appears very late in asexual cycle and is coarser and darker; mature gametocytes larger than size of normal red cell (*vide* Table II).

#### (d) *Plasmodium kochi* var. *macfiei* n. var.

'*Plasmodium inui*'. Macfie (1928).

*Plasmodium* sp. Seidelin and Connal (1914) Connal and Coghill (1916).

*Plasmodium kochi*. Theiler (1930).

Macfie (1928) found a *Plasmodium* in a young *Papio sphinx* at Accra on the Gold Coast which he provisionally referred to as *P. inui*. He observed the animal for 5 months and gives the following description of the parasite, illustrated by 16 black-and-white figures.

*Asexual cycle.* Young rings very large about  $\frac{1}{2}$  diameter red cell; usually single, rather large chromatin mass, but may be two smaller masses one at each diameter of parasite; more mature trophozoites decidedly amœboid, pseudopodia sometimes very fine ('tenué' phase), but more commonly rather blunt and massive; chromatin usually single irregular mass, with adjacent clear vacuole even in very amœboid parasites; no advanced stage schizogony or mature schizonts seen, although many, often daily, blood examinations made; with Leishman's stain cytoplasm grey or faint brown not blue; pigment abundant, fine and light brown, appearing early (figures show large vacuole and chromatin mass in very large rings often with irregular outline and early pigment; more mature forms very amœboid; about half the cells depicted show deformed outline); parasitized cells not at all, or very slightly, enlarged; 'no distinct stippling, but occasionally a few small dots', although special stains used to demonstrate; cells sometimes with indistinct matt or ground-glass appearance.

*Sexual cycle.* Gametocytes like *P. vivax*; usually larger than red cell; mature forms free; pigment abundant and not infrequently appears greenish (figures show indistinct poorly staining nucleus); exflagellation repeatedly observed.

*Pathogenicity.* Heavy infection when first seen; irregular but considerable degree of fever, which subsided in few days and at same time parasites became scanty, but persisted thus for several months; animal seemed to be in a state of tolerance. A chart of temperatures taken twice daily for 11 days is given, but no periodicity

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of fever is apparent. Subcutaneous injection of blood to adult *Cercopithecus mona*, adult *Erythrocebus patas* (*Cercopithecus patas*) and young *Cercocebus lunulatus* failed to infect.

Macfie (1928), while thinking that his parasite would be expected to be *P. kochi* (Lav.), considers that in several points it differs from the descriptions given of the latter. He says the parasitized cells are not enlarged as in *P. kochi*, the pigment granules are fine and of light brown colour; no definite stippling is present like the fine stippling in *P. kochi* and the latter parasite is said to show little signs of pathogenicity, whereas his parasite caused an acute infection.\* He admits, however, that the latter difference may be due to the younger age of his monkey, as compared with the older monkeys infected with *P. kochi*. He considers his parasite as regards stippling and in other respects resembles *P. inui*, so he places it provisionally in this species.

Seidelin and Connal (1914) in Nigeria found two specimens of *Papio sphinz* and one of *Cercopithecus mona*† infected with malaria parasites. These were always scanty and mostly sexual forms, but in two cases a few schizonts were found. No advanced stages of schizogony were seen. Pigmentation was bright yellow in fresh preparations and brilliant green in Giemsa-stained preparations. The chromatin of the gametocytes, and also the protoplasm, stains faintly. Macfie (1928) says he studied a slide from one of the baboons (*Papio*) examined by Seidelin and Connal (1914) and compared it with those of his own parasite. He considers the two parasites identical.

Theiler (1930) reported a malarial parasite in three specimens of *Cercopithecus diana* shot in Liberia. He identifies the parasite with *P. kochi*. The following description is taken from his coloured figures‡ and his account of the parasites:—

**Asexual cycle.** Like *P. vivax*; tendency of infested cell to be enlarged but not markedly so; no stippling present; no fully-developed schizonts seen. [His figures show (a) large ring about 1/3rd diameter red cell with very thin protoplasmic ring around large vacuole with medium-sized, slightly irregular chromatin dot. (b) Parasite about 1/2 diameter of red cell, slightly amoeboid, with large vacuole, oval chromatin dot and little brown pigment. (c) Large, flimsy, amoeboid form, almost filling cell, with fine brown pigment, a vacuole with a long thin, curved, chromatin mass at one side in a distorted red cell. (d) Compact parasite with thick dark-blue pseudopodium separated from the chromatin by a large vacuole, and filled with numerous fine brown pigment granules.]

**Sexual cycle.** A male gametocyte is figured with abundant, fine, light brown pigment, poorly stained protoplasm, with rod-like chromatin dot in clearer area.

**Pathogenicity.** One monkey had a fairly intense infection and showed both sexual and asexual forms, but in other two, only rare gametocytes found.

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\* Macfie (1928) is apparently making a comparison with *P. kochi* of Gonder and Berenberg-Gossler (1908), which is in our opinion a different species from *P. kochi* (Lav.) (vide infra *P. inui* var. *gonderi*).

† These were apparently the same monkeys examined by Connal and Coghill (1916).

‡ From preparations stained with Giemsa's stain (?).

The figures given by Theiler (1930) very closely resemble the figures of '*P. inui*' of Macfie (1928) and the two parasites seem possibly identical.

It can be seen from the above descriptions that the parasites described by Macfie (1928) and by Theiler (1930) bear a considerable resemblance to *P. kochi* var. *joyeuxi* and may possibly be the same parasite, or, if the latter is eventually raised to specific rank, might perhaps be classified as a variety. There are, however, several very definite differences between the various descriptions and figures of *P. kochi* var. *joyeuxi* and *P. kochi* var. *macfei*:—Chromatin in the latter is never so large or so irregular as in the former; young rings are very much larger and have a more voluminous vacuole; pigment appears early, not late, in the asexual forms and is light, not dark, brown in colour; pigment in the gametocytes is greenish, not yellow or dark brown; the distortion of infested cell, which is not at all or only slightly enlarged.

Such variations in morphology may simply be due to the different genera of host from which the parasites were described. Variations within these limits in the same parasite under such conditions have been recorded by Knowles and Das Gupta (1932) in *Plasmodium* sp. found in *Cercopithecus pygerythrus* and transmitted to *Silenus rhesus* (*Macacus rhesus*).

The very rare occurrence of a few dots like stippling in the infested cell suggests that this parasite may be allied to the '*P. inui* group', as Macfie (1928) thought, but this occurrence is much less marked and much rarer than in *P. inui* group. The typical double, markedly unequal, chromatin dots of the latter species are not figured or recorded, nor were segmenting forms found in the peripheral blood, even in the acute infection described by Macfie. The pathogenicity also seems slighter than in the *P. inui* group and all attempts to transmit the infection failed. For these reasons we consider this parasite to be nearer the *P. kochi* than the *P. inui* group.

A differential diagnosis from *P. kochi* and its other varieties is given in Table II.

### Discussion on the '*kochi* group' of malaria parasites.

From the data given, it can be seen that the majority of the Plasmodia which have been described in detail from the genus *Cercopithecus* have a large number of points in common, and in this paper such parasites have all been placed in the '*kochi*' group.

The features common to these parasites are:—

(a) They appear to be the most usual *Plasmodium* in the blood of monkeys of the genus *Cercopithecus*, but they may also occur in the genus *Papio*.

(b) The infection usually appears to cause little inconvenience to the host, but occasionally pathogenic signs are present.

(c) Gametocytes are the commonest forms seen in the peripheral blood.

(d) Schizogony appears to take place in the internal organs; mature segmenting forms are absent from the peripheral blood and asexual forms of any kind may be scanty.

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(e) No enlargement or distinct stippling have been observed in cells infested by asexual parasites.\*

(f) Mature gametocytes are usually free in the plasma and may be larger than the normal red cell, more especially in the case of macrogametocytes.

(g) The nucleus of the trophozoites or the merozoites have never been described or figured as consisting of one large main chromatin dot and a very small accessory one.\* The nuclei of gametocytes are usually large, vesicular and poorly stained, with compact and relatively small amounts of chromatin.

(h) Transmission of the infections to the same or different genera of monkey has so far been unsuccessful.

(i) The duration of the cycle of schizogony is doubtful, but is possibly 48 hours (Martoglio *et al.* 1910).

The above details apply not only to *P. kochi* (Lav.) but also to its three varieties described above (*joyeuxi*, *bowilliezi* and *macfiei*). The points differentiating these three varieties from the type species are given in Table II.

From the summaries given in Table II it can be seen that, while these four parasites have much in common yet as one proceeds from *P. kochi* (Lav.) to *P. kochi* var. *macfiei*, the differences from the type species become greater and greater. These are so marked between the two extremes that it seems to us very possible that at least two species are included under the name *P. kochi*. It may, however, be that these varieties are simply the result of infection by the same parasite in hosts of different species or genera, as have been recorded by Knowles and Das Gupta (1932).

### OTHER MALARIAL INFECTIONS OF AFRICAN MONKEYS REPORTED AS DUE TO *P. KOCHI* (LAV.).

Many infections of African monkeys with *P. kochi* have been reported at different times, in addition to those recorded above. In some instances few or no data have been given, which would make any specific identification possible, while in other cases it seems highly probable that the parasites mentioned are not *P. kochi* (Lav.), but some other species or variety. These records include both natural infections and those produced by blood inoculation from other monkey hosts.

#### Natural infections.

These may be classified according to the genus of the host.

\* It seems to us remarkable that of the many authors, who have identified their parasites as *P. kochi* by a comparison with the descriptions and figures given by Gonder and Berenberg-Gossler (1908) and Berenberg-Gossler (1909), no one seems to have referred to or figured the accessory chromatin dot on which these authors lay so much stress. Again if *P. kochi* of Gonder and Berenberg-Gossler be *P. kochi* (Lav.), it is curious that no other workers have reported stippling or enlargement of the red cells infested by the asexual parasites.



TABLE II.

Parasite.	TROPHOZOITES.			GAMETOCYTES.	
	Chromatin (karyosome).	Young forms.	Older forms.	Size.	Pigment.
<i>Plasmodium kochi</i> (Lav.) 1899.	Very small	Signet-ring; vacuole present; dot-like chromatin.	Some ameboid activity. Vacuole present. Pigment appears fairly early and is abundant fine, yellow or yellowish brown. Chromatin scanty and with little pleomorphism.	Size of normal red cell (?).	Very fine yellowish brown; abundant.
<i>Plasmodium kochi</i> var. <i>boulengeri</i> Leger, 1922.	Very small	Elongated oval not signet-ring; no vacuole; dot-like chromatin.	No tendency ameboid activity. No vacuole. Pigment appears fairly early and in few large, coarse, dark granules. Chromatin scanty and may be dumbbell-shaped.	Larger than normal red cell.	♂ golden yellow; ♀ olive green; very coarse and abundant in both.
<i>Plasmodium kochi</i> var. <i>joyeuxi</i> Leger, 1928.	Large	Ring with elongated chromatin around vacuole.	Very marked ameboid activity, producing 'tenue' forms. Large vacuole. Pigment appears late and is abundant, fine, dark brown or blackish dust. Chromatin abundant and very pleomorphic.	Larger than normal red cell.	♂ yellow; ♀ dark brown; fairly coarse and abundant in both.
<i>Plasmodium kochi</i> var. <i>macfiei</i> n. var.	Medium sized	Very large rings. Chromatin round or irregular inside edge of very large vacuole.	May show marked 'tenue' forms. Large vacuole. Pigment appears early and is abundant, fine and light brown. Chromatin usually single mass of irregular shape.	Larger than normal red cell.	Greenish, abundant, fine.

Genus *Cercopithecus*.

*P. kochi* has been reported as a parasite of monkeys of this genus by many observers. Berenberg-Gossler (1909) records, in two specimens of 'green' *Cercopithecus* (*C. sabæus*), the '*P. kochi*' of Gonder and Berenberg-Gossler (1908). As mentioned previously and as will be discussed later, this parasite is probably not the same species as that described by Kossel (1899). Plimmer (1912) also reports a parasite 'probably *P. kochi*', in the same species of monkey, said to have come from Sierra Leone. Leger and Bouilliez (1913) mention the occurrence of this species of parasite in *C. callitrichus*. Ringenbach (1914) found microgametocytes, with a peripheral nucleus and brownish pigment, in *C. cephus* from the Congo, and considered the parasite to be *P. kochi* (Lav.).

Napier and Campbell (1932) report a malarial parasite in *C. pygerythrus*, and made the tentative suggestion that it might be *P. kochi*. From the detailed description of this parasite given by Knowles and Das Gupta (1932), and from our own studies of the same strain kindly supplied by the latter workers, there is no doubt that this parasite is not *P. kochi* (Lav.) sens. restr., nor any of the varieties described above (vide infra *P. knowlesi* sp. n.). Stiles and Nolan (1929) state that *P. kochi* was reported from *C. pygerythrus* (*erythrotis*) at the London Zoo.\*

Genus *Cercocebus*.

The '*P. kochi*' of Gonder and Berenberg-Gossler (1908) was originally obtained from *C. fuliginosus* and the same strain was used by Berenberg-Gossler (1909) and by Gonder and Rodenwaldt (1910) in their later work. We consider this parasite to be more closely allied to *P. inui* than to *P. kochi* (vide infra *P. inui* var. *gonderi*). Plimmer (1916) reported *P. kochi* in *C. æthiopicus* from Nigeria at the London Zoo, and Grigorieva (1929) gives a description of a parasite, which was considered by him to resemble *P. kochi*, from the same species of monkey.

Genus *Erythrocebus* (*Cercopithecus*).

Stiles and Nolan (1929) state that *P. kochi* has been found in the blood of *E. patas* in French Guinea.\*

Genus *Papio* (*Cynocephalus*).

Koch (1898) and Kossel (1899) have recorded *P. kochi* (Lav.) in *P. cynocephalus* (*babuinus*) from East Africa. While Leger (1928) mentions the occurrence of this parasite in a specimen of *P. sphinx* in French Guinea. In the same species of *Papio*, Grigorieva (1929) found a parasite, said to resemble *P. kochi*.

Genus *Pan*.

Luhe (1906) gives a short description of a malarial parasite found by Ziemann in the chimpanzee. He records this as *P. kochi* (Lav.), but from the

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\* We have been unable to trace the origin of these records, which do not seem to be included in the Zoological Record.

morphology and the natural host of the parasite, it seems to us very unlikely that this identification is correct.\*

Genus undetermined.

Ross (1907) mentions the finding of *P. kochi* in monkeys in Africa. Castellani and Chalmers (1913) say that this parasite causes illness and death among monkeys in Ceylon.

#### Successful inoculation experiments.

Genus *Cercopithecus*.

Napier and Campbell (1932) and Knowles and Das Gupta (1932) transmitted the parasite, which the former authors thought to be possibly '*P. kochi*', to other monkeys of the same species, i.e., *C. pygerythrus*. We have also obtained similar results in the production of chronic infections in this monkey, both with the strain used by the former workers and with another strain obtained by us from the same species of monkey. We, however, consider that this parasite probably belong to the '*P. inui* group' rather than to the '*P. kochi*' one.

Genus *Cercocebus*.

Gonder and Berenberg-Gossler (1908), Berenberg-Gossler (1909) and Gonder and Rodenwaldt (1910) have all succeeded in transmitting the '*P. kochi*' of the first named workers to other specimens of its natural host, *C. fuliginosus*. This, like the parasite previously mentioned, probably belongs to the '*P. inui* group'.

Genus *Callithrix*.

Anderson and Cowdry (1928) report the successful transmission of a parasite resembling *P. kochi* var. *bouilliezi*, found in two specimens to the American 'genus *Callithrix* (closely resembling *C. personata*)' from Senegal, to two other monkeys of this species from the same area.

#### Negative transmission experiments.

The negative inoculation experiments, which have been recorded in describing *P. kochi* and its different varieties, may be summarized as follows:—

Genus *Cercopithecus*.

Joyeux (1913), Bouilliez (1916) and Leger (1926) all failed to transmit *P. kochi* (var. *joyeuxi*) to *C. callitrichus*. Macfie (1928) also failed to infect *C. mona* with his '*P. inui*' (*P. kochi* var. *macfiei*).

Genus *Erythrocebus*.

Bouilliez (1916) and Leger (1928) failed to infect *E. patas* with *P. kochi* (var. *joyeuxi*). The attempts made by Macfie (1928) to infect the same species with his '*P. inui*' were also unsuccessful.

Genus *Cercocebus*.

Macfie (1928) failed to transmit his parasite to *C. lunulatus*.

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\* Luhe (1906) figures a female gametocyte with much darker and coarser pigment than found in *P. kochi* and the young rings have much more chromatin.

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### Genus *Papio*.

Leger (1928) made unsuccessful attempts to infect *Papio* (*Cynocephalus*) sp. with *Pl. joyeuxi*.

### Genus undetermined.

Kossel (1899) was unable to infect other monkeys (sp. ?) with *P. kochi* (Lav.). Leger (1928) was unsuccessful in the transmission of *P. joyeuxi* to a monkey (sp. ?) and Martoglio *et al.* (1910) records similar results with *Silenus* (*Macacus*) sp.

These unsuccessful attempts to transmit *P. kochi* or its varieties to other African monkeys seems to us a strong point in favour of the classification, which we have tentatively made, because parasites of the '*P. inui* group' seem to be easily inoculable into all the genera mentioned, in contradistinction to the '*P. kochi* group'.

## OTHER SPECIES OF *PLASMODIUM* REPORTED FROM THE LOWER PRIMATES OF AFRICA.

In addition to the malarial parasites which we have placed in the '*P. kochi* group', two other *Plasmodia* have been reported which we consider should be regarded, for the time being at least, as separate species or varieties. These two parasites are (a) the '*P. kochi*' of Gonder and Berenberg-Gossler (1908) and (b) the *Plasmodium* sp. of Knowles and Das Gupta (1932). The former parasite, in our opinion, resembles more closely the descriptions of *P. inui* and its varieties than those of *P. kochi*. For this reason we have provisionally named it *P. inui* var. *gonderi*. The parasite recorded by Napier and Campbell (1932) and by Knowles and Das Gupta (1932), we believe to be a previously unnamed species, and for it we propose the name *P. knowlesi* sp. n.

### *Plasmodium inui* var. *gonderi* n. var.

*Plasmodium kochi*. Gonder and Berenberg-Gossler (1908); Berenberg-Gossler (1909); Gonder and Rodenwaldt (1910).

Gonder and Berenberg-Gossler (1908) found and described a *Plasmodium* occurring in the blood of specimens of *Cercocebus fuliginosus* in the Hamburg Zoo. The origin of these animals is not stated, but the natural habitat of the species is West Africa.

Gonder and Berenberg-Gossler (1908) and Berenberg-Gossler (1909) describe their parasite as *P. kochi* (Lav.), but the morphological characters of this *Plasmodium* show considerable differences from the '*P. kochi* group' and many points of resemblance to *P. inui* and its varieties.

These points of resemblance may be summarized as follows :—

- (1) Youngest asexual forms very small, oval or pear-shaped bodies devoid of vacuole.
- (2) Presence of 'accessory chromatin dot' in merozoites and young trophozoites.

- (3) Presence of segmenting forms in peripheral blood.
- (4) Definite changes in infested red cells, e.g., enlargement, stippling and pallor.
- (5) Pigment coarse and rather dark; scanty in segmenting forms but abundant in sexual forms.
- (6) Chromatin in all forms stains deeply and is relatively abundant.
- (7) Easily transmissible to other monkeys.

Monkeys of the genus *Cercopithecus* may be considered as the African representatives of the Oriental genus *Silenus* (*Macacus*) the common natural host of *Plasmodium inui* and its varieties. For this reason the occurrence of a parasite of the '*P. inui* group' in these monkeys is not so extraordinary.

A more detailed account of this parasite will be given when the Plasmodia of the '*P. inui* group' are considered.

#### ***Plasmodium knowlesi* sp. n.\***

*Plasmodium* sp. Franchini (1927).

? *Plasmodium kochi*. Napier and Campbell (1932).

*Plasmodium* sp. Knowles and Das Gupta (1932); Sinton and Mulligan (1932; 1932a).

Franchini (1927) reported a *Plasmodium* in *S. cynomolgus* which he considered to be different from either *P. inui* or *P. cynomolgi*. This parasite has yellowish brown pigment, some mature schizonts were found in the peripheral blood and there was marked deformity of the parasitized cells.

Napier and Campbell (1932) found a *Plasmodium* in the blood of a monkey identified as *Cercopithecus pygerythrus*, purchased in Calcutta and said to have been imported from Singapore. The parasite is described as having 'many of the characters of *P. kochi*'. This strain of *Plasmodium* was investigated by Knowles and Das Gupta (1932) and described in great detail by them. Thanks to the courtesy of the latter workers, we have been able to study this strain of parasite. From our observations we consider it to be more closely allied morphologically to the '*P. inui* group' than the '*P. kochi*' one, because of the presence of an accessory chromatin dot, the schizogony in the peripheral blood, the stippling, etc. We have found a very similar parasite in the blood of two Oriental monkeys, *Silenus pileatus* (*Macacus pileatus*)† as well as in another specimen of the same species of monkey in which Napier and Campbell (1932) discovered their parasite. These three monkeys were purchased in Calcutta

\* Fuller descriptions of this parasite and the reports of the other authors mentioned, will be given later in this paper.

† We are deeply indebted to Dr. Bains Prashad, D.Sc., F.R.S.E., of the Indian Museum, Calcutta, for the identification of this animal.

and, like the original monkey mentioned above, were said to have been imported from Singapore.

Examinations of the bloods of animals infected with this parasite, taken at two or three-hourly intervals, for 24 to 48 hours in several cases, have shown that the cycle of schizogony in this parasite is only 24 hours, not 48 hours, as recorded in other species. Several points in the morphology of the *Plasmodium* are also distinctly different from those recorded in any of the other named parasites of monkeys. There is often marked deformity of the infested cell, like that seen in *P. ovale*, and as figured by Franchini (1927) for his parasite; red cells infested by older parasites have often a shrunken and spinulose appearance. For these reasons we consider this parasite to be a new species, for which the name *Plasmodium knowlesi* is proposed.

It seems to us a very curious coincidence that two monkeys, of which one was identified as an Oriental and the other as an African species, in the same lot of animals, imported from the same place and at the same time, should show the same species of parasite in their bloods. If these reports\* are correct, this suggests to us the possibility that one or both of these animals acquired the infection in some other place than its natural habitat. As Green (1932) has shown that malaria is not uncommon among the monkeys of Malaya and that his species of *Plasmodium* develops freely in some of the common Anophelines of that country, the possibility that one or both these animals acquired the infection at Singapore cannot be neglected. In the meantime the point as to whether *P. knowlesi* is to be considered as primarily a parasite of an African or an Asian species of monkey remains *sub judice*.\*

Because this parasite has also been found in a typically Asian species of monkey, *S. pileatus*, and that it seems to be morphologically more closely allied to the '*P. inui* group', the common Plasmodia of Oriental monkeys, we consider that a detailed description of its morphology, etc., can be more conveniently given with *P. inui*, when the malarial parasites of Oriental monkeys are being dealt with.

(To be concluded).

(Absence of space has prevented the complete inclusion of this article in the current number of these Records. The second portion, with the appendices and references, will appear in the next number.—Ed.).

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\* While this article was in the press we have been informed that the species of monkey identified as *Cercopithecus pygerythrus*, the natural host of *P. knowlesi*, has since been found to be the Oriental monkey *Silenus irus* (*Macacus cynomolgus*). Similarly the monkeys formerly identified for us as *S. pileatus* have since been found by Dr. Bains Prasad to be immature specimens of *S. irus*. In the light of these revised identifications our deductions as to the Oriental character of *P. knowlesi* are verified. *P. knowlesi* may therefore be regarded definitely as an Oriental *Plasmodium*.

A CRITICAL REVIEW OF THE LITERATURE RELATING TO  
THE IDENTIFICATION OF THE MALARIAL PARASITES  
RECORDED FROM MONKEYS OF THE FAMILIES  
CERCOPITHECIDÆ AND COLOBIDÆ

(continued).\*

BY

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PLASMODIA FOUND IN THE BLOOD OF ORIENTAL  
MONKEYS OF THE FAMILIES COLOBIDÆ  
AND CERCOPITHECIDÆ.

IN Oriental countries the occurrence in nature of blood infections with *Plasmodium* has only been recorded in the genus *Pygathrix* (*Semnopithecus* ; *Presbytis*) of the sub-family COLOBINÆ of the former family, and in the genus *Silenus* (*Macacus*) in the sub-family CERCOPITHECINÆ of the latter.

PLASMODIA RECORDED AS NATURAL INFECTIONS IN MONKEYS  
OF THE SUB-FAMILY COLOBINÆ.

Only one species of *Plasmodium* has been reported from this sub-family, namely *P. semnopithecii* Knowles, 1919, from *Pygathrix entellus*.

\*The first part of this review, dealing mainly with the malaria parasites of the lower African monkeys, appeared in the last number of these Records (*Rec. Mal. Survey India*, 3, 2, pp. 357-380).

***Plasmodium semnopithecii* Knowles, 1919.***P. semnopithecii.* Knowles (1919).*Plasmodium* sp. Chimisso (1922).*P. semnopithecii.* Wenyon (1926).

Knowles (1919) discovered a *Plasmodium* in the blood of a hanuman monkey, *Pygathrix entellus*, in Assam. This animal, which had been under observation for about ten months, appeared to be in excellent health. Immediately following upon an injection of 2 c.c. of blood, taken from a patient showing only sexual forms of *P. falciparum* in the peripheral blood, this monkey became seriously ill and died within 48 hours. The blood at the time of death showed a very heavy infection with malarial parasites. Believing this *Plasmodium* to be different from any monkey parasite previously described, Knowles (1919) suggested the name *Plasmodium semnopithecii* for it.

Very little description was given of the morphology of this parasite, but the paper is illustrated by 93 coloured figures. From the description and figures given,\* this *Plasmodium* appears to have the following characters :—

*Appearances in stained preparations.†*

*Asexual cycle.* Youngest forms almost non-pigmented rings, closely resembling those of *P. falciparum*; some rings larger and more flimsy, resembling ring forms of *P. vivax*; double infestations of red cell sometimes seen. (Figures show smallest forms about 1/6th diameter of red cell; may be typical 'signet-ring' forms, or with very small vacuole, compact protoplasm and rounded chromatin dot; larger rings 1/4th to 1/3rd diameter red cell with very large vacuole, thin flimsy protoplasm and small chromatin mass; protoplasm may be slightly thickened at side opposite chromatin, which is single, usually rounded but sometimes oval or rod-shaped; pigment appears as few brown granules when parasite about 1/3rd diameter red cell. Older forms figured are more than 1/2 diameter red cell, with very large vacuole; protoplasm thickened but ring form maintained, sometimes till nearly fills corpuscle; pigment in fine, dark brown granules, sometimes clumped, usually very abundant, but sometimes scanty; little evidence of amoeboidicity; red cells infested with large vacuolated forms appeared distinctly enlarged as compared with those infested with smaller forms; no stippling shown at any stage.)

No segmenting forms seen either during 48 hours of life or at autopsy, although the infection was severe.

*Sexual cycle.* Numerous free gametocytes seen in peripheral blood and smears from spleen. No detailed description given. (Figures show macrogametocytes apparently larger than normal red cell; protoplasm blue; chromatin mass relatively small, oval or elongated, deeply staining and situated close to achromatic area of varying size but usually small; pigment very dark brown and very abundant; individual granules very fine but tend to aggregate into small dark masses. Microgametocytes smaller than females but larger than normal red cell; chromatin large, diffuse and rose-coloured; pigment as in macrogametocytes.)

\*We have relied mainly on the figures of the parasites found in the peripheral blood, as it seems possible that some of the forms seen in the internal organs may be degenerate (Knowles, 1919; Knowles and Das Gupta, 1932).

† Blood films stained with Leishman's stain.



*Schisogony cycle.* Not determined.

*Pathogenicity.* Latent infection apparently awakened by protein shock due to intravenous injection of foreign blood; severe illness, coma, prostration, sub-normal temperature, and death in 48 hours with very heavy parasitic infection; spleen and bone-marrow congested at autopsy.

It is difficult to give the diagnostic characters of this parasite, but, so far as can be judged from the figures reproduced, the most outstanding features are:—early appearance of fine brown pigment; growing forms as large flimsy rings with very large vacuole, very abundant pigment, little amoeboidicity, and single, oval or rod-shaped chromatin mass; absence of segmenting forms in peripheral blood. Macrogametocytes often free and larger than normal red cells; chromatin (karyosome) small, deeply staining, oval or much elongated, and sometimes in relation to unstained or very faintly stained vacuole (?); pigment very dark brown, very abundant and in very fine granules or small dark collections. Microgametocytes smaller than macrogametocytes and with very large pink nuclei.

Chimisso (1922), working in Italy, discovered a *Plasmodium* in the blood of a monkey identified as *Silenus rhesus*. The origin of the monkey was uncertain, but it was thought that it had come from India. During the period while it was under observation, about one month, malarial parasites were encountered in its blood in considerable numbers. The blood was examined on numerous occasions, sometimes as often as twice daily and at least three times a week. From the description and the forty coloured figures which accompany it, this *Plasmodium* appears to have the following characters:—

*Appearances in fresh preparations.*

Very young forms unpigmented and difficult to see. Larger forms pigmented and more easily recognized; pigment yellowish, yellowish brown or almost black. Gametocytes distinguished by spherical shape, larger and more abundant pigment granules, and by size, which exceeds that of normal red cell.

*Appearances in stained preparations.\**

*Asexual cycle.* Youngest forms small, clearly defined rings,  $1/5$ th to  $1/8$ th diameter of red cell, resembling those of *P. falciparum*; thin wisp of clear blue protoplasm surrounding relatively well-developed vacuole; sometimes protoplasm faint and rings appear incomplete; chromatin mass marginal or inside vacuole, deep red, rounded or elongated, usually single but may be double; minute 'accessory chromatin dot' frequently present; no pigment in youngest forms. (Figures indicate forms as described, and show infested red cells unaltered at this stage.)

Larger rings  $1/4$ th to  $1/3$ rd diameter red cell; usually not pigmented, but older forms with scanty or well-marked pigment mostly in dark granules. More advanced forms amoeboid, with vacuole and blue protoplasm; pigment pale yellow, yellowish brown or darkish; red cells frequently decolorized, but no enlargement noted; stippling not demonstrated even with special stains. (Figures show slight irregularity of parasites, but amoeboidicity not marked; some ring forms nearly fill cell; pigment sometimes appears early, often very abundant and may be more marked towards periphery of parasite; vacuolation usually very marked; infested red cells little, if at all, enlarged.) No larger growing forms described. (Figures

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\* Blood films stained with May-Grunwald-Giemsa stain.

suggest that some of the 'crescent-like' forms described as gametocytes may be immature asexual forms; protoplasm clear blue, usually markedly concavo-convex, pointed at one or both ends and thickened in the middle; chromatin small, round, oval or elongated, and often appearing detached, or attached only by a thin rim representing the remains of the red cell; vacuole very marked, separating chromatin from protoplasm; pigment granules often very abundant, fine and varying from yellowish brown to almost black; red cell absent or only shown as slender arc extending between ends of crescentic protoplasm; whole parasite size of, or rather larger than, normal red cell.)

Segmenting forms never observed in peripheral blood, or in smears from liver or spleen, although specially searched for. (Figures show two forms with double chromatin mass, which might be interpreted as early segmenting forms.)

*Sexual cycle.* Gametocytes rounded and closely resembling those of *P. vivax* and *P. malariae*. Macrogametocytes relatively large (8 to 12 microns), with dense deep-blue protoplasm; chromatin small and dense, sometimes with accessory chromatin dot; single mass or aggregation of small, dense granules. [Figures show macrogametocytes free and often larger than normal red cell; protoplasm deep blue; chromatin (karyosome) small, dense, and sometimes surrounded by light pink or clear areola; vacuole sometimes present; pigment often very abundant, occurring in medium-sized granules, varying from yellowish to almost black, and scattered through protoplasm.] Microgametocytes mostly smaller than females; protoplasm stains less deeply and often blue grey or blue green; chromatin nucleus large, occupying 1/3rd to 1/2 entire parasite; sometimes two dense nuclei seen (figures suggest these may be early segmenting forms); pigment very abundant in coarse granules of varying tints, usually brown or blackish. (Figures show vacuole, frequently marked.)

In addition to the forms described above Chimento refers to other types of gametocytes, often in shape of 'crescents'; some of these believed to resemble those of *P. falciparum* ('semilune-simili') with chromatin in protoplasm; other similar forms seen with chromatin detached ('pseudo-semilune'); 'ovoid bodies', said to resemble corresponding forms of *P. falciparum*, also seen (Figures suggest that some of these forms are immature, rounded asexual parasites, and derive their peculiar 'crescentic' shape from the presence of a large vacuole placed excentrically with crescentic mass of protoplasm at one side.)

*Schizogony cycle.* Not determined.

*Pathogenicity.* Only one infected monkey observed; occasional exacerbations of fever noted, but not correlated with development of parasite. Attempts to transmit infection to another monkey of the same species failed. Infected monkey died about one month after time of first observation.

Chimento (1922) has emphasized the similarity between this monkey *Plasmodium* and *P. falciparum*. The points of resemblance which he stresses are:—absence of stippling in infested red cells; absence of segmenting forms from peripheral blood; presence of 'crescent-like' gametocytes, ovoid bodies and small slender rings often with double chromatin mass; frequent decolorization of infested red cells.

After a careful study of Chimento's figures, we are convinced that the various forms of 'crescentic gametocytes', which he describes, are in no way comparable to the crescents of *P. falciparum*, and that these forms are wholly attributable to the presence of a large vacuole in rounded sexual or

asexual forms. It appears to us that some of the figures reproduced and purporting to represent gametocytes, may possibly be immature asexual forms.

In our opinion there is little doubt that the *Plasmodium* described by Chimento (1922) is probably identical with that described by Knowles (1919), as *P. semnopithecii*. A comparison of the figures reproduced by these two authors has shown that an almost exact facsimile of each of Chimento's figures is to be found among those given by Knowles. The only particular in which we have found noteworthy difference is in the presence of an 'accessory chromatin dot' in Chimento's parasite, while this has not been figured by Knowles.

Wenyon (1926) also records *P. semnopithecii* from a specimen of *Presbytes pileatus*\* (sic!) from Assam, examined at the London Zoo by himself and Dr. Scott. No description of the parasite is given.

#### *Discussion on Plasmodium semnopithecii Knowles, 1919.*

Of the parasites which we have included in this group, only two have been described or figured in any detail, namely those of Knowles (1919) and of Chimento (1922); the similarity between these two parasites is remarkable. Wenyon (1926) has expressed the opinion that both these parasites bear a close resemblance to *P. inui*, while Knowles and Das Gupta (1932) think that '*P. semnopithecii* is probably synonymous with some previously-described monkey *Plasmodium*'.

We agree that the descriptions and figures given of these parasites have several features of resemblance to those of the '*P. inui* group' (*vide infra*), but they differ in the following important respects:—

(1) Segmenting forms not found in the peripheral blood, even in heavy infections.

(2) Vacuolation of the parasite may be very marked, even more so than in any of the '*P. inui* group'. This may give rise to 'pseudo-crescents' often larger than a red cell.

(3) Chromatin relatively small in amount and often very much elongated. It may appear detached from the protoplasm on account of the large vacuole.

(4) Gametocytes frequently much larger than red cells and often markedly vacuolated.

(5) The infested red cell may be markedly decolorized in contrast to the hypercoloration seen with *P. inui* infections.

(6) Stippling has never been demonstrated in spite of special efforts.

(7) An attempt to transmit the parasite to another monkey failed.

We are, therefore, of opinion that until more evidence is available, *P. semnopithecii* should not be considered as identical with *P. inui* or either of its varieties. We have also been unable to identify this parasite definitely with

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\* For a discussion of the identity of this monkey, the reader is referred to the foot-note in Appendix I (B) under *Silenus pileatus*.

any of the other *Plasmodia* reported from monkeys, as suggested by Knowles and Das Gupta (1932).

The specific identity of *P. semnopithecii* is supported by the fact that the natural occurrence of *P. inui* in Indian monkeys has not been confirmed\*. It is also noteworthy that, except for Donovan's undescribed parasite,\* the only natural malarial infections which have been reported from Indian monkeys, namely by Knowles (1919), by Chimisso (1922) and by Wenyon (1926), would all appear to be due to *P. semnopithecii*. It seems possible that this species or variety of monkey *Plasmodium* may be peculiar to India.

Until more accurate information is available, *Plasmodia* having the following general characters, especially if of Indian origin, should be classified as *P. semnopithecii* :—

*Asexual cycle*.—Youngest forms, rings 1/6th to 1/5th diameter red cell, closely resembling those of *P. falciparum*. Larger ring forms 1/4th to 1/3rd diameter red cell with flimsy blue protoplasm and large vacuole. Pigment appears very early, when rings about 1/3rd diameter red cell, and is usually dark brown, fine and abundant, but may tend to form small dark masses. Older forms as very large rings with very large vacuole; these may almost fill the infested cell; chromatin relatively small and oval or elongated; many forms extracellular; amœboidicity slight or absent. Some large rings may exceed diameter of red cell; usually with crescentic protoplasm, very large vacuole and sometimes with chromatin apparently detached; pigmentation usually very dense. Decolorization of red cell frequently observed, but stippling never seen. No segmenting forms found in peripheral blood.

*Sexual cycle*.—Gametocytes rounded, usually free and often larger than normal red cell. Macrogametocytes stain deep blue; chromatin (karyosome) small, usually peripheral and often with clear or light pink areola; pigment granules of medium size, very abundant and of variable colour, yellowish to almost black, but usually dark; pigment may be scattered or aggregated into small masses; vacuolation sometimes seen and may be marked. Microgametocytes smaller than females; protoplasm stains less deeply; chromatin large and diffuse and usually rose-coloured; pigment as in females, but may be coarser.

*Pathogenicity*.—So far only recorded with certainty from Indian monkeys of the genera *Pygathrix* and *Silenus*. No record of successful transmission to other monkeys. Infection capable of producing intense illness (*Pygathrix*), but may remain latent or give rise to sub-acute condition (*Silenus*).

#### PLASMODIA RECORDED AS NATURAL INFECTIONS IN MONKEYS OF THE SUB-FAMILY CERCOPITHECINÆ.

Natural infections of the blood with *Plasmodium* have been recorded in many Oriental monkeys of this sub-family, but these animals have all belonged

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\* Donovan (1920) records a *Plasmodium* from the blood of a specimen of *S. sinicus* from South India, but no description is given. The morphology of this parasite is said to resemble the *Plasmodium* described from *S. irus*, presumably *P. inui* or *P. inui* var. *synnolgi*.

to the genus *Silenus*\*. A list of the various records of natural infections in monkeys of this genus, as well as of those of infections transmitted to other monkeys by inoculations from such infected animals, is given in Appendix I. In these numerous records, the parasites found have remained unnamed, or have been identified either as *P. inui* Halberstadter and Prowazek, 1907, or as *P. cynomolgi* Mayer, 1907.

Several authorities have expressed the opinion that *P. inui* and *P. cynomolgi* are the same parasite. Mesnil (1907), after a study of the original descriptions and figures, states that these two parasites are undoubtedly identical. Mathis and Leger (1911), Leger and Bouilliez (1912, 1913) and Wenyon (1926) may be mentioned among those who have expressed a similar opinion.

Blanchard and Langeron (1912) have, on the contrary, taken the opposite view and state most emphatically that *P. cynomolgi* is a valid species and cannot be regarded as identical with *P. inui*. This opinion is based chiefly on two morphological characters, namely stippling, and the nature and amount of pigment produced. Mayer (1908) had previously adopted a similar attitude, but admitted the possibility of identity. Macfie (1928) has pointed out that, in classifying the Plasmodia of monkeys, two characters which may prove of considerable assistance are the nature of the stippling, and that of the pigment produced. This author does not appear to agree that *P. inui* and *P. cynomolgi* are identical.

After a very careful study of the literature we are of the opinion that, while *P. inui* and *P. cynomolgi* are very much alike in their general characters and morphology, the descriptions of these two parasites show certain differences (notably in the size and staining reactions of the infested red cells, and in the nature of the pigment), which cannot be ignored. While we think it probable that *P. inui* and *P. cynomolgi* may eventually prove to be two distinct species, the available data regarding *P. inui* are not sufficiently definite to make the differentiation of these parasites absolutely certain. We also believe that future classification will be facilitated and further confusion lessened, if, for the present, parasites having the morphology given in descriptions of *P. cynomolgi* be recognized as a variety of *P. inui*. We, therefore, propose to classify those parasites, which we consider to conform most closely to the original description of *P. inui*, as *P. inui* Halberstadter and Prowazek, 1907, and those which are, in our opinion, more closely related to *P. cynomolgi*, as *P. inui* var. *cynomolgi* Mayer, 1907.

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\* Considerable diversity of opinion appears to exist as to the correct name of this genus. It is referred to as *Silenus*, *Macacus*, and *Macaca* by different authors. We have, however, followed Stiles and Nolan (1929) in our nomenclature. The correct specific name of the common brown Indian monkey of this genus is also said by some workers to be *mulattus*, not *rhesus*, but we have retained the usual name, pending some more definite decision on this point. There seems, however, to be a consensus of opinion that the monkey formerly called *S. cynomolgus* should be known as *S. irus*, and we have followed this decision.

Earlier in this paper we have referred to the *Plasmodium* described by Gonder and Berenberg-Gossler (1908), and later by Berenberg-Gossler (1909) and Gonder and Rodenwaldt (1910), as *P. kochi*. We have indicated that, in our opinion, this parasite bears a closer morphological resemblance to the '*P. inui* group' of monkey *Plasmodia* than to the '*P. kochi* group', and have proposed the name *P. inui* var. *gonderi* n. var. for it. Although this parasite was originally reported from an African monkey, *Cercocebus fuliginosus*, it is more convenient to consider it at this stage, especially as this species of monkey may be regarded as the African representative of the Oriental genus *Silenus*.

We have also pointed out above that the malarial infection described by Knowles and Das Gupta (1932), and studied by us through the courtesy of the latter workers, appears to be identical with the infections found by us in several naturally infected specimens of *S. irus*, said to have been imported from Singapore.

The parasite described by Franchini (1927) seems to be identical with parasites found by us in the infections mentioned, and named *P. knowlesi* sp. n. This parasite has been described along with the *P. inui* group, to which it has many points of similarity. We think, however, that it might possibly be more suitable if a new group had been created for this species, on account of its 24-hour cycle of schizogony and its peculiar morphology.

### THE '*P. INUI* GROUP' OF MONKEY PLASMODIA.

We propose that, for the present, the monkey malaria parasites which, in our opinion, show many points of resemblance to *P. inui*, and which for the most part have been described from the lower Oriental monkeys, or their African representatives, should be divided into four main divisions as shown below :—

(a) *Plasmodium inui* Halberstadter and Prowazek, 1907 (sens. restr.).

*Plasmodium inui*. Mathis and Leger (1911)

*Plasmodium inui*. Leger and Bouilliez (1912; 1913); Bouilliez (1913)

(b) *Plasmodium inui* var. *cynomolgi* Mayer, 1907.

*Plasmodium cynomolgi*. Mayer (1907; 1908).

*Plasmodium cynomolgi*. Blanchard and Langeron (1912; 1913).

*Plasmodium cynomolgi* (?). Donovan (1920).

*Plasmodium inui* (?) Green (1931; 1932); Kingsbury (1931).

*Plasmodium kochi* (?) (pro parte). Napier and Campbell (1932).

*Plasmodium* sp. (pro parte). Knowles and Das Gupta (1932).

*Plasmodium* sp. (pro parte). Sinton and Mulligan (1932a).

*Plasmodium inui* var. *cynomolgi*. Sinton and Mulligan.

(c) *Plasmodium inui* var. *gonderi* n. var.

*Plasmodium kochi*. Gonder and Berenberg-Gossler (1908); Berenberg-Gossler (1909); Gonder and Rodenwaldt (1910).

*Plasmodium kochi*. Grigorieva (1929).

(d) *Plasmodium knowlesi* sp. n. Sinton and Mulligan, 1932.*Plasmodium* sp. Franchini (1927).*Plasmodium kochi* (?) (pro parte). Napier and Campbell (1932).*Plasmodium* sp. (pro parte). Knowles and Das Gupta (1932).*Plasmodium* sp. (pro parte). Sinton and Mulligan (1932a).*Plasmodium knowlesi*. Sinton and Mulligan (1932b).(a) *Plasmodium inui* Halberstadter and Prowazek, 1907 (sens. restr.).*Plasmodium inui*. Mathis and Leger (1911).*Plasmodium inui*. Leger and Bouilliez (1912; 1913); Bouilliez (1913).

Halberstadter and Prowazek (1907) discovered a *Plasmodium* in the blood of *Silenus irus* (*M. cynomolgus*) in Java, and of *S. nemestrinus* in Sumatra and Borneo. To this parasite they gave the name *P. inui*.

Unfortunately their description of this *Plasmodium* is not a detailed one, and consists mainly of references to the ten coloured figures which accompany that portion of their paper which refers to *P. inui*. The characters of *P. inui* as obtained from the description and figures\* given by these workers may be stated as follows:—

**Asexual cycle.** Youngest forms seen were not described (figure shows small solid-looking parasite about 1/4th diameter red cell, composed of relatively broad protoplasmic ring enclosing small vacuole; chromatin single, round and excentric). Reference made to larger extracellular, markedly vacuolated forms showing early division of chromatin (two such forms figured). (Another figure shows parasite with elongated protrusion of protoplasm; although this is evidently early growing form it shows abundant, fine, light brown pigment granules); other similar forms said to have been seen. Division of chromatin early; pigment in dividing forms aggregated in clumps. Mature schizont with 12 to 16 merozoites (figure shows brown pigment in mature schizont as single, rounded mass at centre); (discrete merozoites figured as rounded, oval or pear-shaped bodies; chromatin single dot). Parasitized red cells apparently unaltered at all stages; no changes suggested in description or figures; stippling never observed although demonstrated in *P. pitheci* described at the same time. No additional forms seen in smears from internal organs.

**Sexual cycle.** Macrogametocytes free; nucleus peripheral with distinct inner dark, and lighter outer, zone (figure shows roughly rounded body with protoplasm darker than in microgametocyte; pigment in fine golden yellow granules scattered through protoplasm; chromatin excentric and shows two zones). Microgametocytes—protoplasm stains lightly; nucleus large and peripheral, and rich in chromatin; pigment abundant, fine, yellowish and scattered (figure shows roughly quadrilateral, extracellular body with large deeply staining nucleus, faint protoplasm, and abundant, fine, golden yellow pigment granules).

*P. inui* distinguished from *P. pitheci* by fainter staining of protoplasm, and appearance of abundant, fine, yellowish pigment granules; stippling never observed and younger forms more solid-looking.

**Schizogony cycle.** Not determined.†

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\* Blood films stained with Giemsa's stain.

† The periodicity of this parasite has frequently been quoted as 48 hours. The original, however, only states that schizogony, which was commencing when quinine was injected, proceeded uninfluenced for forty-eight hours.

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*Pathogenicity.* No apparent disturbances in health of animals infected in nature. Transmission of the infection was possible to other monkeys of the genus *Silenus* (? same species), but not to orang-outangs.

Mathis and Leger (1911), working in Tonkin, examined the bloods of forty monkeys (*S. rhesus* and *S. lasiotis tcheliensis*) and five of these were found to be infected with a *Plasmodium* resembling that of human malaria. A full account of the morphology of this parasite is given, but from which species of natural host is not indicated. The description is accompanied by forty coloured figures. These authors classify their parasite as *P. inui* Halberstadter and Prowazek, 1907, with which they believe *P. cynomolgi* Mayer, 1907, to be identical. According to the description and figures given, this parasite appears to have the following characters :—

*Appearances in fresh preparations.* Parasites seen as small, round, oval or amoeboid bodies. Youngest forms not pigmented. More advanced forms show pigment in fairly large ('assez gros') and very motile granules; pigment in still larger forms only slightly motile. Exflagellation observed.

*Appearances in stained preparations.\**

*Asexual cycle.* Youngest forms round or oval rings about 3 microns diameter, composed of thin wisp of clear blue protoplasm surrounding relatively large vacuole; chromatin large, excentric, ruby-red dot often more than 1 micron in diameter; two chromatin masses of equal or unequal size common in one ring, sometimes close together and sometimes widely separated (figures do not show any extreme disparity in size of two chromatin dots, i.e., no 'accessory chromatin dot' *vide infra*); double infection rare; rings not pigmented and closely resemble those of *P. falciparum*; infested red cells unaltered. Growing forms pyriform, oval, triangular or semi-spherical; amoeboid forms also seen (figures show evidence of only very slight amoeboidicity); band forms as in *P. malariae* exceptional; protoplasm stains clear blue; pigment in very fine granules ('grains en fine poussière') giving parasite almost greenish tint; chromatin arc-shaped or elongated circle with clear centre; vacuole of very variable size; infested red cells unaltered in size; stippling resembling Schuffner's dots sometimes seen with Leishman's stain. Division of chromatin early; forms about 5 microns across with two or more nuclei; division of nucleus by successive and not simultaneous segmentation; vacuole of variable size sometimes present; pigment in fine, greenish yellow granules with tendency to aggregate into single mass. Mature schizont with up to 16 merozoites, usually resembling daisy-head of *P. malariae*; free merozoites composed of large red nucleus and slender rim blue protoplasm; infested red cell not enlarged; stippling occasionally seen but inconstant.

*Sexual cycle.* Adult macrogametocytes free; typically round or slightly oval; larger than mature schizonts, measuring 7 to 8 microns; protoplasm homogeneous and dense stains more deeply than in schizonts; vacuole sometimes present; irregular pigment granules coarser than in trophozoites; pigment granules greenish brown, scattered but more abundant at periphery; chromatin small, excentric with darker inner zone, and outer faint pink or unstained zone. Immature gametocytes have same characters but are intracellular; nucleus less compact and often have large vacuole which disappears as reach maturity. Microgametocytes extracellular, irregularly rounded and smaller than macrogametocytes (about 6 microns);

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\* Blood films stained with Leishman's and Giemsa's stains.



protoplasm ash-blue; pigment lighter, less abundant, and in larger granules than in macrogametocytes; pigment granules often of unequal size; nucleus larger, less dense, and often shows as pink mass with some more deeply staining granules.

Stippling when seen is inconstant; only demonstrable with Leishman's stain and never with Giemsa's.

*Schizogony cycle.* Not definitely determined; blood examinations and fever curves in inoculation infections suggestive of 48-hour cycle.

*Pathogenicity.* No symptoms in natural infections, which can only be determined by blood examinations. Infection often prolonged and shows tendency to spontaneous cure. Transmission to other monkeys of genus *Silenus* easy (? same species); some febrile reaction observed in inoculation infections, but symptoms not severe.

Mathis and Leger (1911) identify this parasite as *P. inui* Halberstadter and Prowazek, 1907. The only difference noted was the occasional occurrence of stippling, resembling Schüffner's dots, in some of the infested red cells. This was never seen when Giemsa's stain was used, which was the stain used by Halberstadter and Prowazek (1907).

Leger and Bouilliez (1913) have given a detailed description of a *Plasmodium*, previously found by them (Leger and Bouilliez, 1912) in the heart blood of a specimen of *S. irus* (*M. cynomolgus*) among the experimental animals at the Pasteur Institute, Paris. The original monkey which was found to be infected, was one of a batch of five of the same species, all of which died shortly after their arrival at the Institute. The strain was, however, maintained by sub-inoculation to other monkeys, and formed the subject of two papers (Leger and Bouilliez, 1912; Bouilliez, 1913) before the detailed description given by Leger and Bouilliez (1913) was published. Unfortunately the latter account is unaccompanied by figures, but from the very full description given, the characters of this *Plasmodium* may be summarized as follows:—

*Appearances in fresh preparations.* Parasites in red cells as small, round, oval or amoeboid bodies; youngest forms not pigmented and difficult to see; more advanced forms recognized by motility of pigment; gametocytes distinguished by larger, more numerous, and very actively motile pigment granules; gametocytes and merozoites only free forms seen.

*Appearances in stained preparations.\**

*Asexual cycle.* Youngest forms as very small rings (1.75 microns), composed of thin wisp of blue protoplasm around relatively well-developed vacuole; chromatin ruby-red, usually rounded and always conspicuous; sometimes peripheral and sometimes inside vacuole; chromatin may occur as two masses close together or wide apart (no mention made of any disparity in size of two chromatin masses or of any 'accessory chromatin dot'); double and even triple infection seen, depending on severity of infection; no pigment in young forms. Growing forms amoeboid, more rarely compact or band-like; protoplasm clear blue; pigment scanty or absent; vacuole usually well developed; chromatin always abundant, position and shape very variable, often rod-like, arc-shaped, etc. More advanced growing forms rounded up; pigment more abundant and in fine dust-like granules ('fine poussière pigmentaire') giving parasite greenish tint. Division of chromatin

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\* Leishman's stain mostly used, but good preparations also obtained with Laveran, Giemsa, and iron-haematoxylin methods.

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commences before parasite fills red cell and proceeds until 12 to 16 secondary nuclei produced, scattered indiscriminately at periphery. Mature schizont-like rosette, often smaller than red cell, and with 12 to 16 merozoites; pigment aggregated in mass towards centre.

Infested red cells never enlarged at any stage of asexual cycle, even when two large parasites in one cell; infested red cells sometimes stain more deeply than normal. Stippling resembling Schüffner's dots occasionally seen with both Leishman's and Giemsa's stains; no apparent connection between stippling and age of parasite; Maurer's dots never seen.

*Sexual cycle.* Macrogametocytes often quadrilateral and little larger than microgametocytes; protoplasm stains deeply; pigment granules fine and scattered; chromatin rounded, stains deeply, and shows no vesicular areola. Microgametocyte rounded or polyhedral; protoplasm greyish blue; pigment granules grouped in irregular scattered masses; chromatin diffuse occupying about 1/4th parasite and shows more deeply staining rods lying in lighter pink zone. Gametocytes almost always intracellular, but infested cells not enlarged; stippling resembling Schüffner's dots seen in few instances with both Leishman's and Giemsa's stains.

*Schizogony cycle.* 48 hours; determined by successive blood examinations.

*Pathogenicity.* Very pronounced pathogenic characters, even in natural infections. Transmission successful to *S. irus*, *S. sinicus*, *S. rhesus*, *S. nemestrinus*, *Cercop. callitrichus*, *Erythrocebus patas* and *Papio anubis*. Two types of disease noted, (a) acute, and (b) chronic, but intermediate forms also seen.

Acute form of disease—severe pernicious algid attacks, resulting in death of majority of inoculated animals within 10 days of appearance of parasites. Hæmoglobinuria seen in one *C. callitrichus*, and hæmaturia in one *S. rhesus*.\* Virulence of strain apparently unaltered after 17 passages. Quinine, even in large doses, appeared to have no influence on infection.

Chronic form of disease—characterized by successive relapses and spontaneous cure. This form encountered in 9 out of 24 animals. Virulence apparently independent of age and species of monkey, and not affected by dosage of parasites or route of inoculation.

Attempts to transmit the infection to *Cercocebus fuliginosus*, the chimpanzee, and the 'maki' of Madagascar failed.

Leger and Bouilliez (1913) considered this parasite to be identical with *P. inui* Halberstadter and Prowazek, 1907, which they believe to be the same parasite as *P. cynomolgi* Mayer, 1907.

\*The resemblance of these pathogenic effects to those produced by *P. knowlesi* (*vide infra*), suggested that the two parasites might be identical. Apart from other considerations the definite 24-hour cycle of *P. knowlesi*, as compared with the 48-hour one in *P. inui* of Leger and Bouilliez (1913), clearly differentiates the two parasites. On the other hand, the parasite described by Leger and Bouilliez (1913), was sub-passaged through several specimens of *S. irus* (*M. cynomolgus*) before its hæmoglobinuric effects were produced. The latter species of monkey has been found to be frequently infected with *P. knowlesi* in nature. The possibility that the infection became a mixed one of *P. inui* and *P. knowlesi* during these sub-passages, cannot therefore be ruled out. As both *P. inui* and *P. inui* var. *cynomolgi* have been found by most workers to produce a comparatively mild type of infection, such a mixed infection would account for the hæmoglobinuria mentioned.

*Discussion on P. inui Halberstadter and Prowazek, 1907, sens. restr.*

The three parasites which we have classified above under this name appear to us, after a very careful study of the descriptions and figures, to be identical.

The youngest forms in all three parasites appear as rings. Mathis and Leger (1911) and Leger and Bouilliez (1913) state that these rings resemble those of *P. falciparum*. No description of the ring forms has been given by Halberstadter and Prowazek (1907), but the reader is referred to a single figure showing a small ring form, the characters of which conform to some of those described by the authors mentioned above. The size of the ring forms appears to be about 1/3rd or less the diameter of the red cell. The chromatin is said to be prominent, and the vacuole usually well developed. Both Mathis and Leger (1911) and Leger and Bouilliez (1913) note that the chromatin in the young forms may be, and commonly is, double. The former authors state that the two chromatin masses may be of equal or unequal size, but this point is not mentioned by the latter authors, although their description is a very detailed one. It appears to us extremely unlikely that anything in the nature of an 'accessory chromatin dot' could have escaped the notice of all the above-mentioned workers, when one considers the prominence given to this feature in the descriptions and figures of other earlier workers such as Mayer (1908), Flu (1908), Blanchard and Langeron (1912), and Gonder and Berenberg-Gossler (1908) in their studies of the parasites of monkey malaria. No pigment was recorded in the young ring forms of *P. inui* in any instance.

The growing forms of all three parasites have many features in common. The pigment in all appears early, is golden yellow to brown in colour, occurs in very fine granules, and, in the more advanced forms, is very abundant. Amœboidity of these forms is apparently not marked. One figure given by Halberstadter and Prowazek (1907) suggests slight motility of the parasite. Mathis and Leger (1911) refer to three figures as showing amœboid forms, but these forms are regular, elongated and vacuolated. Leger and Bouilliez (1913) state that the young forms of their parasite are amœboid and of very variable shape, while the more advanced forms are rounded up. No figures are given.

Segmentation of the chromatin in all three parasites commences early, and may be seen even in those forms which are markedly vacuolated. This vacuolation is a prominent feature of all three parasites, and is well shown in the figures reproduced by Halberstadter and Prowazek (1907) and Mathis and Leger (1911). It is also mentioned by Leger and Bouilliez (1913). Marked vacuolation of these forms is one of the points of resemblance between *P. inui* and *P. semnopitheci* to which we have already referred.

The mature schizonts of these three parasites are very similar, and contain from 12 to 16 merozoites in the form of a rosette with a central pigment mass.

The sexual forms of all three parasites are much alike, but some minor differences are recorded. Halberstadter and Prowazek (1907) figure a macrogametocyte as free, roughly quadrilateral, and about the size of a normal red

cell. The description of the macrogametocyte given by Mathis and Leger (1911) agrees in every respect with the figure given by Halberstadter and Prowazek (1907), except that the size is said to exceed very slightly that of a normal red cell, and that, typically, it is round or oval. Leger and Bouilliez (1913) describe the macrogametocyte as being usually intracellular, and never exceeding the size of a normal red cell. The microgametocytes of all three parasites appear to be very similar. Both Leger and Bouilliez (1913) and Mathis and Leger (1911) refer to a tendency for the pigment granules in the microgametocytes to be of unequal size, while Halberstadter and Prowazek (1907) figure the pigment in fine, scattered granules of uniform size.

In no instance has any reference been made to enlargement of the infested red cells at any stage of asexual development in any of these parasites, nor do the figures suggest that this occurs. Leger and Bouilliez (1913) emphasize this point, and state it is possible to find two large parasites in one cell without its undergoing enlargement. Pallor of the infested red cells has not been reported in any of these parasites, and its occurrence is not suggested in the figures. On the contrary there is some suggestion that the reverse is the case, and Leger and Bouilliez (1913) state that the infested cells may stain more intensely than normal ones. Stippling of the infested cells has rarely been observed. Using Giemsa's stain, stippling was never seen in *P. inui* infections by Halberstadter and Prowazek (1907), although they had no difficulty in demonstrating this character in *P. pitheci* examined at the same time. Stippling resembling Schüffner's dots was occasionally observed by Mathis and Leger (1911) in blood films stained with Leishman's stain, but was not seen when Giemsa's stain was employed. Faint stippling is shown in only three of the forty figures given with their paper. Leger and Bouilliez (1913) were able to demonstrate stippling resembling Schüffner's dots, in only a small proportion of cases. This stippling was demonstrated with both Leishman's and Giemsa's stains, but no connection between the presence of stippling and the age of the parasite was observed.

Of these three parasites the only one in which the duration of the schizogony cycle was definitely determined was that described by Leger and Bouilliez (1913), who were able to demonstrate a 48-hour cycle by successive blood examinations. Mathis and Leger (1911) suspected a similar periodicity in their parasites, both from blood examination and from the tendency of the fever curves of inoculated animals to be tertian in type. Halberstadter and Prowazek (1907) apparently did not determine the schizogony cycle of their *Plasmodium*, but from their remarks as to the influence of quinine treatment on the course of the infection, some workers have apparently inferred that the cycle of this parasite is also a 48-hour one.

Some differences are noted in the pathogenic action of these three parasites. The Plasmodia described by Halberstadter and Prowazek (1907) and by Mathis and Leger (1911) did not appear to exert any adverse effects on the monkeys which they found infected in nature, nor did these authors report any

symptoms of a severe character in their experimentally inoculated animals. Leger and Bouilliez (1913), on the contrary, were eventually dealing with parasites the pathogenicity of which cannot be doubted, for death resulted in the majority of the inoculated animals, but, as previously mentioned, the possibility of mixed infections cannot be excluded with certainty.

In spite of minor differences as indicated above, it appears to us that the three parasites, which we have grouped above under the name *P. inui* Halberstadter and Prowazek, 1907, sens. restr., undoubtedly belong to the same species. Until more work has been done, we consider that only those Plasmodia which have the following general characters should be classified under this name :—

*Asexual cycle*.—Young forms appearing as small rings  $\frac{1}{3}$ rd or less diameter red cell, and closely resembling those of *P. falciparum*; chromatin relatively large, single or double, but disparity in size of the two dots not extreme; vacuole well developed, especially in larger rings; no pigment in young rings; infested red cells not enlarged; no stippling, but infested cells sometimes appear darker than normal. Growing forms may be slightly amoeboid at first, but later become rounded up; pigment appears early and increases with growth; pigment granules abundant, fine, yellowish brown, often giving parasite greenish tint; vacuolation often marked but tends to disappear with growth. Segmentation of chromatin appears early; infested red cells not enlarged and show no pallor; stippling usually absent, but fine stippling resembling Schüffner's dots occasionally seen. Mature schizont in form of rosette with central pigment clump; maximum number of merozoites 16.

*Sexual cycle*.—Gametocytes free or intracellular, about size of normal red cell or very slightly larger (7 to 8 microns). Macrogametocytes rounded or roughly quadrilateral; protoplasm deep blue; pigment in abundant, fine, yellowish brown granules, evenly scattered through protoplasm or sometimes more numerous towards periphery; chromatin (karyosome) excentric, small and compact, sometimes with more faintly staining areola. Microgametocytes usually rather smaller than females; often roughly rounded or polyhedral; pigment may be lighter and less abundant than in females; granules fine and scattered, sometimes irregular in size; nucleus large ( $\frac{1}{4}$ th to  $\frac{1}{3}$ rd of entire parasite), stains lightly and may show few darker chromatin granules.

*Schizogony cycle*.—48 hours.

*Pathogenicity*.—Variable. May be accompanied by few, or no symptoms in natural host. Very acute infections with algid, pernicious attacks, hæmoglobinuria and death have been recorded in inoculated animals.\* Easily transmissible to monkeys of the genus *Silenus*. Leger and Bouilliez (1912; 1913) record experimental transmission to animals of the genera *Cercopithecus*, *Erythrocebus* and *Papio*. Failure to infect the orang-outang, the chimpanzee and the 'maki' by blood inoculation.

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\* Vide foot-note, p. 392.

**(b) *Plasmodium inui* var. *cynomolgi* Mayer, 1907.***Plasmodium cynomolgi*. Mayer (1907; 1908); Flu (1908).*Plasmodium cynomolgi*. Blanchard and Langeron (1912; 1913).*Plasmodium cynomolgi* (?). Donovan (1920).*Plasmodium inui* (?). Green (1931; 1932); Kingsbury (1931).*Plasmodium kochi* (?) (pro parte). Napier and Campbell (1932).*Plasmodium* sp. (pro parte). Knowles and Das Gupta (1932).*Plasmodium* sp. (pro parte). Sinton and Mulligan (1932a).*Plasmodium inui* var. *cynomolgi*. Sinton and Mulligan.

Mayer (1907), working in Hamburg, described as *P. cynomolgi* a *Plasmodium* discovered by him in the blood of a specimen of *S. irus* (*M. cynomolgus*), which had been imported from Java. This author (Mayer, 1908) gave a further description and figures of the same parasite in a later paper. The characters of this parasite as obtained from his descriptions and figures are as follow :—

***Appearances in stained preparations.\****

**Asexual cycle.** Youngest forms, small rings closely resembling those of *P. falciparum*; protoplasm narrow and slender; chromatin mass rounded and prominent; when infestation double, rings sometimes elongated and often marginal; chromatin sometimes double and in addition often see one or two very small, light red, 'accessory chromatin dots'; red cells unaltered; (figures show earliest forms very small and devoid of vacuole; larger forms with well-developed vacuole; no pigment in young forms).

In growing forms protoplasm broadens and shape very variable; often amoeboid but less so than in corresponding forms of *P. vivax*; pigment absent or in scanty golden or brownish granules; (figures show forms markedly amoeboid and without pigment; infested red cells pale and appreciably larger than normal; stippling resembling Schuffner's dots constant with larger forms; no separate 'accessory chromatin dot' seen at this stage). Division of chromatin sometimes seen in amoeboid forms; later dividing forms rounded up; pigment scanty and begins to aggregate into larger masses. Adult schizonts with 8 to 13 merozoites, irregularly placed; pigment scanty and aggregated into dark brownish mass at centre or periphery of the parasite; (figure shows enlargement and marked pallor of infested red cell; also pronounced stippling).

**Sexual cycle.** Macrogametocytes free or intracellular; resembling those of *P. vivax*, and often larger than normal red cell; protoplasm deep blue and sometimes vacuolated; chromatin compact and usually peripheral, sometimes with inner darker area (karyosome); pigment more abundant than in asexual forms; (figures show pigment in fairly large yellowish brown granules; red cell, if present, enlarged and stippled). Microgametocyte free or intracellular; larger than normal red cell; protoplasm less deeply stained; nucleus peripheral or central, often elongated and rich in chromatin, but with no inner dark area (karyosome); red cell, if present, pale and markedly stippled.

**Schizogony cycle.** 48 hours in established inoculation infections.

**Pathogenicity.** No symptoms in natural infections. Transmission to other monkeys by inoculation was successful with *S. irus*, *S. rhesus* and *Cercopithecus* sp.

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\* Blood films stained with Giemsa's stain.

*Insect transmission.* Attempts to infect *Aedes aegypti* (*Stegomyia calopus*) and *Culex pipiens* failed, but young oöcysts were found in *An. maculipennis*.

Flu (1908) made an independent investigation of the same parasite as that studied by Mayer. His description adds little to that given above. Flu noted definite enlargement and pallor of the infested red cells containing the larger growing forms of parasite. Stippling was constantly observed in such cells. This author emphasized the resemblance between this parasite and the form of *P. vivax* seen in patients from Java. The figures reproduced show the following points:—

Earliest form, solid and devoid of vacuole; this form resembles the free merozoite and contains an 'accessory chromatin dot'; two discrete merozoites figured show an 'accessory chromatin dot'. Growing forms amoeboid with scanty or no pigment; infested red cells enlarged and show stippling; pigment darker than figured by Mayer (1908). Attempts to infect *An. maculipennis* failed

Blanchard and Langeron (1912) found a *Plasmodium* in the blood of a specimen of *S. irus* (*M. cynomolgus*), which had been purchased in Europe for experimental purposes. Blood from this monkey was inoculated into another monkey of the same species, which acquired a heavy infection. The description of the parasite given refers to the appearances seen in this inoculated animal. From the detailed account and the accompanying 47 coloured figures, the characters of this *Plasmodium* would appear to be:—

*Appearances in stained preparations.\**

*Asexual cycle.* Youngest forms rings 3 to 5 microns diameter, composed of thin wisp of blue protoplasm surrounding clear vacuole; chromatin variable in shape and position—sometimes marginal giving 'signet-ring' appearance, sometimes crescentic; chromatin may be double, two masses being isolated or connected by thin filament of chromatin; 'accessory chromatin dot' frequently seen ('punctiforme beaucoup plus petite'); double or treble infestation sometimes seen; pigment absent or at most one or two granules; infested red cells unaltered. More advanced forms, large rounded masses of blue protoplasm with 2, 3 or 4 nuclei; pigment granules abundant, coarse and very dark; amoeboid forms seldom seen; stippling or red cells constant; (figures show stippling and slight, but definite, enlargement of red cells with larger parasites; pallor of red cells neither mentioned nor figured; some parasites show very moderate amoeboidity; pigment black, scanty and diffuse); secondary nuclei rounded, compact and well defined. Adult schizonts with 8 to 13 merozoites, irregularly scattered as in *P. vivax*.

*Sexual cycle.* Macrogametocyte large globular, almost filling red cell; protoplasm deep blue; pigment granules large, dark and abundant; (figures show pigment granules black, scanty and not very coarse); chromatin usually excentric, small, staining deeply and uniformly; parasite usually inside red cell showing marked stippling. Microgametocytes free or intracellular; protoplasm stains pinkish; chromatin large and diffuse, often peripheral and sometimes elongated; gametocyte may show reddish capsule.

Text states that infested red cells undergo no alteration in size; this is not borne out by figures, which show slight but definite enlargement in those drawn to same scale. Pallor of red cells is neither mentioned nor figured.

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\* Blood films stained by Pappenheim's panoptic method.

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**Schizogony cycle.** 48 hours; determined by successive blood examinations.

**Pathogenicity.** Variable. Transmitted to two specimens of *S. irus*, one of which acquired a mild chronic infection and other died of acute infection. These authors consider severity of infection may depend largely upon whether the animal has previously suffered from the disease.

Blanchard and Langeron (1913) record the results of experiments carried out by Bouinol, who died before the results of his work were published. Bouinol worked with the same species of monkey and with the same strain of parasite as that described by Blanchard and Langeron (1912). From his figures and notes, published by Blanchard and Langeron (1913), the following *additional* characters have been obtained :—

Very amœboid forms seen in splenectomized animals; sometimes the number of merozoites increased to 15 or 16 after splenectomy, but other apparently fully developed schizonts with only 4, 5, 6, 7 or 8 merozoites also seen; [figures (from measurements) show considerable enlargement of some parasitised cells, even from non-splenectomized animals; distinctly amœboid forms also seen in latter animals]; Schüffner's dots constant with larger forms; schizogony cycle constantly 48 hours in both normal and splenectomized monkeys (*S. irus*); (figures show scanty medium-sized, almost black, pigment granules in all large parasites); pathogenicity in experimentally infected *S. irus* very variable in all animals examined, usually very mild.

Donovan (1920) examined a large number of animals, 76 *S. sinicus* and 10 *Pygerythrus priamus* (*Presbytis priamus*), in the Nilgiri Hills, South India, but found none infected with malarial parasites. Subsequently he obtained a blood film from a specimen of *S. sinicus* from the same region, in which he found a *Plasmodium*. No description of this parasite is given, but it is stated to be morphologically identical with that found in *S. irus* (*M. cynomolgus*). From the meagre data given, it is impossible to make any certain identification of this parasite, but it is probably one of the '*P. inui* group'.

Green (1931) published a preliminary note on a *Plasmodium* discovered in the bloods of specimens of *S. irus* caught in Malaya. This is the same parasite as was mentioned by Kingsbury (1931). In a later communication (Green, 1932), a short description and 20 coloured figures of this parasite are given. From these the characters of this *Plasmodium* would appear to be :—

*Appearances in fresh preparations.* Parasite very actively amœboid.

*Appearances in stained preparations.\**

**Asexual cycle.** Small rings resembling those of *P. falciparum* occasionally seen; these associated with larger rings and growing forms; red cells containing larger growing forms show pallor, enlargement and stippling similar to Schüffner's dots. Mature schizonts with 8 to 16 merozoites. (Figures show rings 1/4th to 1/3rd diameter red cell; vacuole large and pale, surrounded by slender wisp blue protoplasm; chromatin marginal, prominent, sometimes double; 'accessory chromatin dot' sometimes seen; red cells usually unaltered. Growing forms irregularly amœboid,

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\* Blood films stained with one of 'panoptic' methods, i.e., Leishman's stain, undiluted for 30 secs., followed by twice the volume of diluted Giemsa's stain (1 drop to 1 c.c.) and allowed to act for 20 mins. Water used for diluting the stains was buffered with phosphates.



resembling corresponding forms of *P. vivax*; vacuole usually well marked; pigment yellowish-brown or darker granules of moderate size; red cells containing larger parasites enlarged, pale and constantly stippled. Dividing forms rounded up; vacuole disappears; pigment not abundant, aggregated in more advanced forms; infested red cells enlarged, pale and stippled. Mature schizonts larger than normal red cell; maximum number of merozoites 16; pigment scanty in few large, dark grains.)

*Sexual cycle.* Macrogametocytes more abundant; protoplasm deep blue; infested cells enlarged and stippled. (Figures show these forms intracellular; larger than normal red cell; protoplasm deep blue; no vacuole; excentric nucleus, smaller than in male forms; pigment in medium-sized, evenly scattered granules and not very abundant; infested cells enlarged, pale and stippled.) Microgametocytes less common; protoplasm stains purplish red as in *P. vivax*. (Figures show nucleus larger and less deeply stained than in female forms; nucleus peripheral or central; pigment similar to that in macrogametocytes).

Only distinguishing character found by careful comparison with *P. vivax* was smaller maximum number of merozoites (max. 16) in monkey *Plasmodium*.

*Schizogony cycle.* Not determined.

*Pathogenicity.* Natural infections found only in young monkeys (*S. irus*); 3 animals found infected out of 12 examined. No apparent symptoms in natural infections. Infection easily transmissible by blood inoculation to monkeys of same species, producing bouts of fever followed by splenic enlargement and perhaps anaemia. Later, chronic infections developed with some evidence of immunity and tolerance.

*Sporogony cycle in mosquitoes.* Oöcysts and sporozoites obtained in *Anopheles kochi*, *An. maculatus* and *An. vagus*; these indistinguishable from those of *P. vivax*. No development found in *An. philippinensis*, *An. aconitus*, *Armigeres obturans*, *Culex fatigans* and *Aedes albopictus*.\*

Green (1932) provisionally classified this *Plasmodium* as *P. inui* Halb. and Prow., but from his figures and brief description, it appears to us to have more points of resemblance to *P. inui* var. *cynomolgi* Mayer. As the cycle of schizogony has not been mentioned in any of the reports on this *Plasmodium*, we referred to Dr. Green for further information on this point. He informs us that he has been unable to determine the duration of this cycle and has very kindly sent us some stained blood films showing his parasite.† From an examination of these, we are of opinion that there is little doubt

\* This appears to be the first successful attempt to obtain the complete sporogony cycle of any of the Plasmodia found in the Old World monkeys, although Clark and Dunn (1931) have succeeded with the Plasmodia of the New World ones.

Mayer (1908), working with *P. cynomolgi*, got no development in *Stegomyia calopus* (*Aedes aegypti*) or in *Culex pipiens*, but found young oöcysts in *Anopheles maculipennis*. Flu (1908), with the same parasite, failed to obtain development in the last-named mosquito. Berenberg-Gossler (1909) also failed to infect this mosquito with *P. inui* var. *gonderi*. Macfie (1928) tried to infect *Anopheles gambiae*, *Mansonioides africanus* and *Aedes argenteus* (*Aedes aegypti*) with *P. kochi* var. *macfie*, but without success.

† One of the slides received showed some parasites, which suggested that, while the predominant infection was *P. inui* var. *cynomolgi*, a very scanty infection with *P. knowlesi* was also present. This was shown by the morphology of some of the Plasmodia and by the characteristic deformity of the infested cells at some stages. The latter feature is not seen with *P. inui* var. *cynomolgi* in our experience.

that the *P. inui* described by this worker is identical with *P. inui* var. *cynomolgi* Mayer, although confirmatory evidence of the duration of the schizogony cycle is still lacking.

Another *Plasmodium*, morphologically indistinguishable from that described above, was found by Green (1932) in the blood of a young female monkey, identified as *S. irus mordax* and believed to have been imported from Java.

Quite recently we have been successful in obtaining from a mixed infection of *P. knowlesi* and *P. inui* var. *cynomolgi*, what we consider to be a pure infection with the latter parasite. This strain was first isolated in a specimen of *S. rhesus* inoculated from a specimen of *S. irus* found infected in nature. The latter monkey was said to have come from Singapore.

Through four sub-passages in *S. rhesus*, this parasite has remained true to type (*vide* Appendix II). It is hoped to give a fuller and illustrated description of this parasite in a later paper. As will be seen from the description given below, it is undoubtedly identical with *P. inui* var. *cynomolgi* Mayer, 1907.

#### *Appearances in stained preparations.\**

*Asexual cycle.* Youngest forms occur as small, solid, non-vacuolated bodies about  $1/6$ th to  $1/5$ th diameter of red cell, consisting of chromatin dot or blob and wisp of blue protoplasm; these forms resemble merozoites. Slightly older forms (1-3 hours) appear as rings  $1/5$ th to  $1/3$ rd diameter red cell, closely resembling those of *P. falciparum*. Protoplasm seen as very delicate, regular, hair-like ring surrounding well-developed vacuole. Chromatin dot prominent, usually single, round or oval, frequently projecting from ring giving classical 'signet-ring' appearance; chromatin may be within vacuole and may be rod- or arc-shaped rather than round. Two chromatin dots in one ring sometimes seen, the two masses being of approximately equal size, and situated either close together or wide apart. Sometimes the two chromatin masses may show great disparity in size—one large dot and one very minute accessory one; latter may be variable in position but frequently it is situated within vacuole, close to protoplasmic ring, and at some distance from main dot. Rings usually excentrically placed in red cell but true accolé forms rare. Double infestation of one cell occasionally seen. Infested cells unaltered at this stage.

Slightly older forms (3-6 hours) show marked irregularity in shape, protoplasm being stretched out into very fine irregular threads; sometimes ring form more or less retained, but protoplasm often shows localized irregular thickenings or protrusions of one or more fine elongated processes. No pigment observed in these forms, but infested cells often begin to show evidence of commencing changes such as slight enlargement, hypercoloration or very fine, almost imperceptible stippling.

On further growth (6-9 hours) parasites less irregular and tend to reassume ring form. These rings measure  $1/3$ rd to  $1/2$  diameter red cell. Protoplasm shows small localized thickenings (pseudopodia) or distinct regular thickening at pole opposite chromatin. No pigment present. Infested red cells

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\*Blood films stained by the panoptic method described by Green (1932) and with Giemsa's stain. Unless where otherwise stated, the appearances here described refer to those seen in panoptic films.

show slight but definite enlargement, and hypercoloration and stippling is more pronounced.

When  $1/2$  or rather more the diameter red cell (12–15 hours), protoplasm of parasite is thicker and coarser and more regular in outline, giving appearance of large, coarse ring or slightly 'tailed' form. Chromatin larger and sometimes shows clear area at centre; accessory dot not seen in larger forms. Some irregularly amoeboid forms seen but not common. Pigment absent, or at most one or two very fine, yellowish brown grains. Enlargement and stippling of infested red cells constant.

Older trophozoites (18–30 hours) closely resemble those of *P. vivax* but amoeboidity is rather less marked. Some retain the ring form, measuring  $1/2$  to  $2/3$ rd diameter red cell or even more. Chromatin round or oval, separated from main bulk of protoplasm by vacuole which is, at first, large but which tends to become relatively smaller with growth. Protoplasm markedly thickened, especially opposite chromatin, and often appears crescent-shaped or 'tailed', but may be more irregularly amoeboid. Pigment is seen in fine, golden yellow, or light brown granules, which are more numerous at periphery of protoplasm but usually not very abundant. The minute accessory chromatin dot seen in younger forms is no longer apparent; in some parasites the presence of one or two more deeply staining dots in the main chromatin mass suggests that the accessory dot may have entered the main mass. Infested red cells show very marked changes—enlargement is constant, closely approximating to that seen in *P. vivax*; stippling resembling Schüffner's dots is well marked and constant, so that infested cells stand out prominently. In lightly stained films stippling may not be seen and the infested cells show marked pallor.

Before segmentation of chromatin commences (33–36 hours), parasite occupies a large proportion of infested cell. Vacuole is much reduced or absent and parasite has solid compact appearance like that of immature gametocyte. Chromatin is usually single, round or oval, and peripheral. Pigment is more abundant and occurs in moderately fine golden brown to dark brown granules, which are more numerous towards periphery. Infested red cells much enlarged and stippling very intense; in some cases contour of cell not distinct and recognized only by intense stippling.

Early segmenting forms (36–40 hours) almost entirely fill the red cells. Parasite appears as a dense, solid mass of protoplasm with from 2 to 6 chromatin nuclei; vacuole absent, or one or more very small vacuoles may be seen. Pigment tends to aggregate into coarse brown or dark brown granules.

Mature schizonts (48 hours) almost fill the infested red cell and exceed slightly the size of a normal red corpuscle. Maximum number of merozoites counted was 16. Pigment is aggregated into single very dark brown or almost black mass, which may be situated either centrally or peripherally; sometimes several smaller masses may be seen of a similar colour. Infested red cells show intense reddish stippling; the dots may be discrete but frequently it appears as if they had coalesced and collapsed on the surface of the parasite, producing the appearance of a red rim or capsule, the outline of which may be irregular giving the parasite a spinulose appearance.

**Sexual cycle.** Gametocytes usually intracellular but may be free. Macrogametocytes are more numerous than microgametocytes. The former, when mature, occur as large round, oval or elongated bodies almost invariably appreciably larger than a normal red cell; protoplasm stains deep blue and is rarely vacuolated. Chromatin most often peripheral, but occasionally may be more centrally placed; chromatin compact and stains deeply (in lightly stained films a less deeply

staining areola is seen surrounding a darker inner zone). Pigment is moderately abundant and occurs in medium-sized granules varying from golden brown to almost black in colour and scattered irregularly through the protoplasm. Infested red cells, when present, much enlarged and show very prominent stippling; a red capsule similar to that described in mature schizonts may be present.

Microgametocytes are rounded or oval bodies exceeding the size of a normal red cell, but often rather smaller; usually less numerous than macrogametocytes. Protoplasm stains indistinctly and is frequently of a reddish purple colour. Chromatin nucleus large and diffuse, and may occupy 1/3rd of entire parasite; it is situated either at the centre or the periphery, but is more rarely seen as an equatorial band. Pigment is similar to that seen in female forms but sometimes the granules are more irregular in size and distribution. Infested cell, if present, shows intense reddish stippling or, as in macrogametocytes, may appear as a deep red capsule surrounding the parasite.

The characters of this parasite bear a remarkable resemblance to *P. vivax*. *Schizogony cycle.* 48 hours. Determined by successive blood examinations. Blood slides were taken at frequent intervals (at least every three hours) day and night for several days. It was found possible to follow the development of the parasite stage by stage in these serial films. In some instances, the schizogony cycle was particularly well demonstrated, practically all the parasites present at a given time being at the same stage of development.

*Pathogenicity.* In natural infections of *S. irus* no symptoms were apparent and the infection was detected only by blood examinations. In inoculation infections in *S. rhesus* moderately heavy infections were obtained, but in spite of this the animals appeared to suffer little or no inconvenience. In some the infection was followed by a marked anaemia. Recovery without treatment resulted in every case.

*Sporogony cycle in mosquitoes.\**

In our opinion the parasite described above is identical with *P. inui* var. *cynomolgi* Mayer, 1907. As has been mentioned above, we were successful in obtaining this parasite in a pure form from a specimen of *S. irus*, which we had previously found infected in nature (Sinton and Mulligan, 1932a) and which later work has convinced us was suffering from a mixed infection (*vide* Appendix II).

As will be mentioned later in this paper, Napier and Campbell (1932) and Knowles and Das Gupta (1932) recorded a malarial infection in *S. irus*, also said to have been imported from Singapore. The description of this infection, as seen in the natural host, *S. irus*, given by the latter workers, has very many characters in common with *P. inui* var. *cynomolgi*. When, however, this infection was inoculated into healthy specimens of *S. rhesus*, a parasite with

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\* Since this paper went to press, the sporogony cycle of *P. inui* var. *cynomolgi* has been obtained in a number of mosquitoes. Sporozoites have been found in the salivary glands of *An. annularis* (*An. fuliginosus*), *An. splendidus* (*An. maculipalpis*), *An. maculatus*, and *An. culicifacies*.

The infection has also been transmitted to a healthy specimen of *S. rhesus* by the bites of infected *An. annularis*. This is apparently the first occasion on which any monkey malaria parasite has been transmitted by the mosquito. No changes in the morphological characters of this parasite could be detected after insect transmission.

a very different morphology was found. In our opinion there is very little doubt that these workers were dealing with a mixed infection of *P. inui* var. *cynomolgi* and *P. knowlesi*, the former parasite being the predominating infection in the natural host, *S. irus*. On inoculation into *S. rhesus* the predominant, and probably the only infection was *P. knowlesi*. This matter has been discussed more fully under *P. knowlesi* and in Appendix II.

*Discussion on P. inui* var. *cynomolgi* Mayer, 1907.

The descriptions of all the parasites which we have grouped under this head bear, in our opinion, a very close resemblance to each other, and there is little doubt that they are all identical. There are, however, several points in relation to the described morphology of these parasites which require some discussion.

(a) *Size of infested red cells*.—In the course of their descriptions of this parasite, several authors have stated definitely that there is no enlargement of the infested red cells, or at most only very slight enlargement of the cells parasitized by the larger growing forms. In spite of these statements the figures reproduced by most of these workers show, in many instances, slight and sometimes considerable enlargement of the red cells infested by larger trophozoites. We have made a careful study of these figures and have estimated the size of the infested red cells as compared with normal ones. In every instance we have found that a considerable proportion of such red cells show an appreciable degree of enlargement. The figures reproduced by Mayer (1908) and by Flu (1908) show clearly that this enlargement may be quite considerable; it approximates very closely to that depicted by Green (1932), and that observed by us for this parasite. The figures given by Blanchard and Langeron (1912) also show this, but to a less extent. Drawings of the same parasite made by Bouinol (Blanchard and Langeron, 1913) show a somewhat greater and more constant degree of this condition, even when the forms encountered in splenectomized animals are excluded. In our experience, the enlargement of the infested red cells in *P. inui* var. *cynomolgi* approximates closely to that seen in human infections with *P. vivax*.

(b) *Stippling*.—Stippling resembling Schüffner's dots has been seen, at some stage of development, in all the Plasmodia included by us under this variety, and, in the majority of cases, this feature has been constant in the cells parasitized by the larger forms. In our own work with this parasite we have been able to demonstrate stippling clearly with both Giemsa's and Leishman's stains, and it has been particularly striking in films stained by the 'panoptic' method used by us.

(c) *Pigment*.—In the descriptions of the parasites which we have grouped as *P. inui* (sens. restr.), emphasis has been laid on the presence of very fine, abundant pigment granules. The pigment granules in *P. inui* var. *cynomolgi* have, on the contrary, been described as being definitely coarser and usually darker than in the corresponding forms of *P. inui* (sens. restr.). Pigment also

appears to be less abundant, especially in the asexual forms but also in the sexual forms of *P. inui* var. *cynomolgi*.

(d) *Amœboidicity*.—Amœboidicity of the earlier growing forms appears, on the whole, to be distinctly more apparent in the parasites of this variety than in *P. inui*. This character has not been observed by Blanchard and Langeron (1912), but very amœboid forms of the same parasite are figured by Bouinol (Blanchard and Langeron, 1913) in splenectomized monkeys, and even in non-splenectomized animals. These figures suggest a greater degree of amœboidicity than has been indicated by Blanchard and Langeron (1912). During our observations on this parasite we have encountered very irregularly amœboid forms but, on the whole, this feature is rather less evident than in *P. vivax* infections.\*

In addition to the points discussed above a striking feature common to all the parasites of this variety of *P. inui* is the presence of a minute 'accessory chromatin dot' in many of the merozoites and young ring forms. This character has not been figured or mentioned in regard to any of the parasites which we have classified as *P. inui* sens. restr.

The mature gametocytes are usually, if not always, distinctly larger than normal red cells, while those in *P. inui* are figured and described as being about the same size as such cells.

The chief differential characters, which serve to distinguish the parasites which we have classified as *P. inui* from those classified as *P. inui* var. *cynomolgi*, are summarized in Table III.

We suggest, therefore, that Plasmodia found in the lower Oriental monkeys and having the following characters, should, for the present, be grouped under the name *P. inui* var. *cynomolgi*.

*Asexual cycle*.—Young rings 2 to 5 microns diameter, round or oval and having prominent chromatin dot and well-developed vacuole; chromatin may be double or treble, often showing great disparity in size, i.e., a minute 'accessory chromatin dot' (rarely two) present; two or three parasites sometimes seen in same red cell in heavy infections; infested cells usually unaltered; no stippling. Growing forms often show pronounced amœboidicity, but usually less marked than in *P. vivax*; pigment appears at about 18 hours in growing forms and is scanty in all asexual parasites; pigment granules yellowish-brown to almost black; darker and coarser than in *P. inui*; vacuolation, especially in large forms not marked; infested red cells appreciably enlarged and pale except with youngest parasites; stippling resembling Schüffner's dots conspicuous. Segmenting forms common in peripheral blood; mature schizonts with 8 to 16 merozoites irregularly scattered; pigment in small dense clump; red cell with marked pallor and prominent stippling.

\* Amœboidicity is most pronounced about six hours, and again about thirty hours, after schizogony. As schizogony frequently takes place about mid-day, amœboid forms might not be evident in films taken in the morning only.

TABLE III.

	Plasmodia classified as <i>P. inui</i> Halberstadter and Prowasek, 1907.	Plasmodia classified as <i>P. inui</i> var. <i>cynomolgi</i> Mayer, 1907.
Infested red corpuscles.	Never enlarged, even if two large parasites in one cell; pallor not seen; may stain more deeply than normal.	Enlargement and pallor* often marked with larger forms of parasite.
Stippling.	Absent or only rarely demonstrable; never constant.	Present; constant with larger parasites
Chromatin.	May be double in ring forms, but no minute accessory dot described.	Minute accessory dot frequently seen in merozoites and young rings.
Pigment.	Very abundant in both asexual and sexual forms; appears early; granules very fine and light in colour (golden yellow to brown).	Less abundant, especially in asexual forms; appears later; granules coarser and darker.
Amœboidicity.	Not marked in young growing forms.	May be pronounced, resembling that seen in <i>P. vivax</i> .
Vacuolation of growing forms.	Marked vacuolation very common in growing forms, even in dividing forms.	Not so marked; absent or very small in dividing forms.
Gametocytes.	About size of normal red cell.	Larger than normal red cell.

\* This pallor is seen when either Giemsa's or Leishman's stains are used, but with the panoptic method the very conspicuous nature of the stippling makes it less apparent.

*Sexual cycle.*—Gametocytes free or intracellular. Macrogametocytes round or oval and usually larger than normal red cell; protoplasm deep blue; chromatin small and compact, excentric and may show lighter-staining areola; pigment not very abundant and granules darker and coarser than in *P. inui*; infested cell, if present, enlarged and stippled. Microgametocytes smaller than macrogametocytes; protoplasm stains less intensely; chromatin central or peripheral, larger and less deeply staining than in macrogametocyte.

*Schizogony cycle.*—48 hours.

*Pathogenicity.*—Variable. May be severe in inoculated animals. Infection inoculable to other lower monkeys (*Silenus* and *Cercopithecus*).\*

(c) *Plasmodium inui* var. *gonderi* n. var.†

*P. kochi*. Gonder and Berenberg-Gossler (1908); Berenberg-Gossler (1909); Gonder and Rodenwaldt (1910).

*P. kochi*. Grigoriewa (1929).

Gonder and Berenberg-Gossler (1909) discovered a *Plasmodium* in the bloods of a number of specimens of the African monkey, *Cercocebus fuliginosus*,

\* Sporogony, vide foot-note on p. 402.

† The reasons for the inclusion of this parasite of African monkeys among the Oriental Plasmodia have already been discussed

which they examined in the Hamburg Zoo. Berenberg-Gossler (1909) found, what he believed to be the same parasite, in monkeys of the same species as well as in two 'green' *Cercopithecus* (*C. sabaeus*). The original habitat of these monkeys is not stated, but it may be inferred that they came from Africa where these species are indigenous. Gonder and Berenberg-Gossler (1908) have given a detailed description of their parasite which they considered to be *P. kochi* (Lav.). From this description, which is illustrated by 33 coloured figures, and from a further description with 39 coloured figures given by Berenberg-Gossler (1909), the chief characters of this *Plasmodium* as seen in *C. fuliginosus*, may be summarized as follows:—

*Appearances in fresh preparations.* Younger forms actively amoeboid; parasites intracellular; pigment granules present in larger forms and resemble those of *P. vivax*, but are coarser and more granular; pigment differs also from that of *P. vivax* in being doubly refractile with polarized light; pigment actively motile and brownish yellow or greenish in colour. Mature schizonts appear as 'daisy-heads' with central clump of brownish pigment. Pigment in sexual forms abundant and very motile.

*Appearances in stained preparations.\**

*Asexual cycle.* Youngest forms very small, solid, oval or pear-shaped bodies about 1/6th diameter red cell; quickly assume ring form; rings about 1/4th diameter red cell with well-developed vacuole; chromatin in ring forms, as well as in earliest solid forms, often double; the two chromatin masses often show great disparity in size—one large chromatin mass and a minute 'accessory dot'—either isolated or connected together by a thin strand of chromatin. (Figures mostly show infested cells unaltered at this stage, but one with ring form shows enlargement, and fine stippling.) Ring forms soon become amoeboid; nucleus increases in size and shows tendency for early mitosis; pigment appears and increases with growth, occurring as scattered greenish yellow-brown granules; less abundant than in sexual forms; 'accessory dot' of chromatin tends to disappear with growth; amoeboid forms at first vacuolated, but vacuole disappears as division of chromatin advances. (Figures show distinct enlargement and fine stippling of red cells infested by trophozoites about 1/4th to 1/2 or more diameter of red cell; some such cells inclined to be oval.) Division of chromatin proceeds until 6 to 16 daughter nuclei formed; pigment tends to aggregate, giving protoplasm brownish colour, but may be scanty. Mature schizonts with maximum of 16 merozoites; chromatin stains deeply; pigment aggregated into several masses; (figures show pigment in variable amounts, usually in scattered masses); discrete merozoites often show a minute 'accessory chromatin dot' in addition to the main chromatin mass, and these forms appear to be youngest asexual forms; other merozoites with single chromatin mass believed to be precursors of sexual forms.

*Sexual cycle.* Youngest sexual forms believed to be those merozoites with single chromatin nucleus showing two nucleoli. Sexual forms characterized by early appearance of pigment, which is coarse and abundant in larger forms. Developing sexual forms show very pronounced vacuole, which disappears later; (figures show marked vacuolation in forms about 3/4th diameter of red cell; some of these forms closely resemble some figures given by Chimiwo, 1922). Mature gametocytes either free or intracellular, and often larger than normal red cell. Macrogametocyte stains deep blue; protoplasm homogeneous; nucleus shows more deeply staining

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\* Preparations stained with Giemsa's stain.



inner, and more faintly staining outer, zone; pigment granules brown and coarse. Microgametocyte stains less deeply; protoplasm alveolar; pigment yellowish green, and granules finer and lighter than in female forms; nucleus large and rich in chromatin and appears in stained preparations as dark red with violet granules. (Figures show one young gametocyte with distinct stippling of infested red cell); exflagellation of microgametocytes figured by Berenberg-Gossler (1909).

Infested red cells.—Enlargement of infested cells often observed, but less marked than in *P. vivax*. Stippling resembling Schuffner's dots frequently seen in cells parasitised by larger growing forms in experimental infections, but was rarely observed in natural infections.

*Schizogony cycle.* At first not accurately determined and appeared to vary from 24 to 50 hours. Later Gonder and Rodenwaldt (1910) were able to show definite tertian periodicity.

*Pathogenicity.* Gonder and Berenberg-Gossler (1908) found this parasite to be easily inoculable to other monkeys (*Cercocebus fuliginosus*), but in only two of these were febrile symptoms observed. Berenberg-Gossler (1909) and Gonder and Rodenwaldt (1910) obtained heavy infections in inoculated monkeys of the same species, but the animals did not appear to be ill. Attempts to inoculate man with this *Plasmodium* failed (Berenberg-Gossler, 1909).

*Sporogony cycle in mosquitoes.* Attempts to infect *An. maculipennis* were unsuccessful (Berenberg-Gossler, 1909).

It is on the above detailed descriptions of the '*P. kochi*' of Gonder and Berenberg-Gossler, and on the excellent plates which accompany them, rather than on the meagre original description of *P. kochi* (Lav.) given by Kossel (1899), that many subsequent workers appears to have based their identifications of the latter species. This seems to have been responsible for much of the confusion which has arisen in the literature.

It will be observed from the summary description given above, that this parasite, although of African origin, differs very considerably from the descriptions and figures of all the other Plasmodia described from Africa, and which have already been dealt with under the '*P. kochi* group'. At the same time this *Plasmodium* appears to us to have many of the characters of the parasites which we have included in the '*P. inui* group'. For this reason we have considered it more suitable to include it with the latter group. The creation of a separate variety of *P. inui* for this parasite has been thought necessary, as we do not consider it advisable at this stage to include a *Plasmodium*, having apparently a different geographical distribution and a different genus of host, among the Oriental Plasmodia, which have been grouped under the name *P. inui* var. *cynomolgi*.

The chief points of resemblance to *P. inui* var. *cynomolgi*, and of distinction from the parasites of the '*P. kochi* group', may be briefly summarized as follows :—

- (1) Youngest asexual forms very small, solid, pear-shaped bodies, later assuming ring shape.
- (2) Presence of 'accessory chromatin dot' in young forms.
- (3) Presence of segmenting forms in the peripheral blood.

- (4) Changes in infested red cells, e.g., enlargement and stippling sometimes seen.
- (5) Coarse and rather dark pigment granules, more marked in the sexual forms.
- (6) Chromatin relatively abundant and dark staining.
- (7) Infection can be transmitted to other lower monkeys.

Grigorieva (1929) has reported the finding, on one occasion only, of a *Plasmodium* in the blood of a specimen of *Cercocebus aethiopicus* at the Moscow Zoo. He gives a very brief description, without figures, of this parasite :—

*Asexual cycle.* Young forms like *P. vivax*; sometimes as small well-shaped rings with central chromatin, but mostly as large irregular rings. Latter with chromatin inside ring and of diverse shapes—ellipsoidal or pear-shaped; sometimes large chromatin mass with 'accessory chromatin dot' on opposite side of ring; accessory dot may later join larger chromatin and form pear-shaped mass mentioned. Larger forms nearly fill cell; very fine pigment.

*Sexual cycle.* Mature gametocytes rare, not larger than normal red cells; usually females. Macrogametocytes with bluish-green protoplasm and peripheral nucleus, which is sometimes compact, round or ellipsoidal, sometimes like loose ball of string; pigment coarse, greenish-brown and evenly scattered or central. Microgametocytes with rose-yellow protoplasm; nucleus at or near periphery, pale pink with loose central mass of chromatin; pigment coarse yellowish-brown at periphery or around nucleus.

No enlargement or stippling of infested cells detected.

*Schizogony cycle.* Not determined.

*Pathogenicity.* Infection only found on one occasion in one specimen of *Cercocebus aethiopicus*; other examinations during one month negative. Mild infection; few symptoms.

Grigorieva (1929) states that this parasite recalls *P. kochi*, presumably that of Gonder and Berenberg-Gossler (1908), in its morphology and the character of its pigment. He was, however, unable to draw any definite conclusions about its identity, without further experimental evidence, for he thinks that the morphology of monkey Plasmodia may vary under different conditions.

The close resemblance between many points in Grigorieva's description of this parasite and *P. inui* var. *gonderi*, as well as the genus of the natural host, *Cercocebus*, suggest that these two parasites are identical. The absence of any recorded enlargement or stippling in the infested red cells is against such a view. When, however, one considers that the parasite was only seen on one occasion and that stippling of the infested cells is rarely seen in natural infections with *P. inui* var. *gonderi*, Grigorieva may not have observed these characters. This parasite is, therefore, placed provisionally with *P. inui* var. *gonderi*.

It is very interesting that these are the only two parasites, found as natural infections of the genus *Cercocebus*, of which we have any description, and that these descriptions should have such a considerable resemblance to

each other. It is very possible, however, that *P. inui* var. *gonderi* may eventually prove to be identical with *P. inui* var. *cynomolgi*.

In his original description of *P. kochi* (*P. inui* var. *gonderi*) in *Cercocebus fuliginosus*, Berenberg-Gossler (1909) also reports the occurrence of *P. kochi* in *Cercopithecus sabaeus*. His description of the former parasite is based entirely on the forms seen in *C. fuliginosus*, so it is impossible to say whether these two parasites were the same. It seems possible that the parasite of *C. sabaeus* may have been *P. kochi* (Lav.) and not *P. inui* var. *gonderi*, for it was in this species of monkey that the former parasite was originally found.

**(d) *Plasmodium knowlesi* sp. n.** Sinton and Mulligan, 1932.

*Plasmodium* sp. Franchini (1927).

*Plasmodium kochi* (?) (pro parte). Napier and Campbell (1932).

*Plasmodium* sp. (pro parte). Knowles and Das Gupta (1932).

*Plasmodium* sp. (pro parte). Sinton and Mulligan (1932a).

*P. knowlesi* sp. n. Sinton and Mulligan (1932b).

In the first part of this paper we proposed the name *P. knowlesi* for a *Plasmodium* found in a specimen of *S. irus* infected in nature, and probably originating in Malaya. This *Plasmodium* was found to be inoculable to *S. rhesus* and to produce very severe pathogenic manifestations in this species of monkey. Attention was drawn to the following characters of this parasite :— (a) a 24-hour cycle of schizogony; (b) marked deformity of many of the infested red cells recalling that seen with *P. ovale*, and a spinulose appearance of the red cells infested by the more mature forms of the parasite; (c) the occurrence of segmenting forms in the peripheral blood; (d) the presence of an 'accessory chromatin dot'; (e) the presence of stippling in some of the infested red cells; and (f) the ease of obtaining successful inoculation infections in *S. rhesus*. It was pointed out that the 24-hour cycle of schizogony, and the characteristic deformity of the infested red cells, were points of marked distinction from any of the other named Plasmodia of monkeys. As the characters of this parasite had many points of resemblance to those of '*P. inui* group' a full description was deferred until the parasites of the latter group were being considered.

Considerable difficulty has been experienced in determining the exact morphological characters of this *Plasmodium*, as seen in natural infections in *S. irus*. This has been attributable to the fact that, in the latter species, the parasites are very scanty, and also that the monkeys available for study were usually suffering from mixed infections, both *P. knowlesi* and *P. inui* var. *cynomolgi* being present.

When specimens of *S. rhesus* are inoculated from monkeys (*S. irus*) found infected in nature and showing mixed infections, the resultant infection in the inoculated animals is, in the vast majority of cases, one in which *P. knowlesi* is the only detectable parasite. After many sub-inoculations we have isolated what we believe to be an absolutely pure strain of *P. knowlesi*, which we have re-inoculated into *S. irus* (vide Appendix II).

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The salient characters of *P. knowlesi* as seen in *S. rhesus*\* are given below :—

### *Appearances in stained preparations.†*

*Asexual cycle.*—The youngest parasites seen occur in the form of small, solid, non-vacuolated bodies  $1/5$ th to  $1/4$ th the diameter of the infested red cell, and consisting of a prominent chromatin dot, and a small wisp or blob of blue protoplasm (Plate V, fig. 1). Very quickly a vacuole develops and the parasite assumes the ring form. These rings closely resemble those of *P. falciparum*, but are usually less delicate, and measure from about  $1/3$ rd to almost  $1/2$  the diameter of the infested cell. Almost invariably the protoplasm shows some slight, but definite, thickening on the side opposite the chromatin; the vacuole is fairly well developed (Plate V, figs. 2 to 7). The chromatin is prominent and occurs either as a single, round, oval, or elongated mass (Plate V, figs. 2, 3, 6 and 7), or in two (rarely three) smaller masses of approximately equal size. When double, the two masses may be situated either close together (Plate V, fig. 4), or at opposite poles of the vacuole (Plate V, fig. 6). As a rule the chromatin projects from the contour of the ring giving the parasite the classical 'signet-ring' appearance, but it may be situated within the vacuole and when so placed it is not infrequently curved and elongated. A very characteristic appearance frequently seen is the presence of one, or rarely two, minute 'accessory chromatin dots', in addition to the main chromatin mass. The position of this minute dot is very variable, but most often it is situated within the vacuole, close to the protoplasmic ring and at some distance from the main chromatin mass (Plate V, figs. 3 and 5). The rings are usually excentrically placed in the cells, but true accolé forms are rare except in heavy infections. No pigment is observed at this stage, and the infested red cells appear to be normal in size, shape, and staining reaction.

With further growth (3 to 6 hours) the rings become coarser. In some a few small rounded pseudopodia may be seen, giving the protoplasm a slightly irregular appearance (Plate V, fig. 8). These rings measure about  $1/3$ rd to  $1/2$  the diameter of the infested red cell. Very irregular forms are seldom seen. In some of the larger forms, the granular appearance of the protoplasm suggests the presence of a very fine pigment dust, but discrete grains are not seen. A very characteristic feature of this *Plasmodium*, which begins to appear at this stage, is the peculiar distortion observed in many of the infested red cells

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\*The description of the morphology of *P. knowlesi* in *S. rhesus* has been given here, because the heavier infections seen in this species and the larger amount of material available, facilitate the study of minute details of morphology. No noteworthy differences were detected between the morphology of *P. knowlesi* as seen in *S. rhesus* as compared with pure infection in *S. irus*.

†Blood films stained with Giemsa's stain and the panoptic method described by Green (1932). The appearances here referred to, are those seen in films stained by the latter method unless otherwise stated.

(Plate V, figs. 8 to 11). This distortion recalls that seen with *P. ovale* (i.e., oval, pear-shaped, fimbriated, triangular or irregularly crenated forms). No enlargement of the infested cells is noted; some even appear to be definitely diminished in size.\* No change in the staining reaction of infested red cells is noted at this stage.

When a little older (6 to 9 hours) the rings become more solid in appearance, the vacuole being relatively small or, in some cases, entirely absent. At this stage the parasite measures  $1/2$  or rather more the diameter of the infested cell, and many show a few very fine granules of golden brown pigment. The characteristic distortion of the infested cells is now well marked (Plate V, figs. 12 to 15).

Later forms (9 to 12 hours) measure about  $2/3$ rd the diameter of the red cell, and occur as solid, rounded bodies with a prominent, excentric chromatin mass, little or no vacuole, and dense blue protoplasm (Plate V, figs. 16 to 19). Very occasionally slightly amoeboid forms may be seen. Pigment is now more abundant and tends to be distributed towards the periphery of the protoplasm. The 'accessory chromatin dot' so characteristic of the earlier forms, is no longer visible as such, but the presence of a more darkly staining area in the main nucleus of some parasites suggests that it may have united with the main mass of chromatin. The infested red cells are frequently distorted; they remain unaltered in their staining reaction, and show no evidence of increase, but rather of diminution, in size.

On further growth (12 to 15 hours) the same general morphology is maintained, but the parasite has increased slightly in size, the pigment is more abundant and the individual granules are darker and coarser (Plate V, figs. 20 to 23). Slight pallor of some of the infested red cells may be observed at this stage, but the peculiar distortion referred to previously is still the most striking abnormality.

Before segmentation of the chromatin commences (15 to 18 hours) the parasite may almost fill the infested cell. The majority of forms seen at this stage are large, solid, non-amoeboid, non-vacuolated bodies with a single, large chromatin mass situated laterally (Plate V, figs. 24, 25 and 26). Pigment is now coarser and more abundant, the colour of the granules varying from greenish brown to almost black.† As in the earlier stages, the pigment granules are more numerous towards the periphery of the protoplasm. The peculiar distortion of the infested red cells is still much in evidence, and, in blood films stained with Giemsa's stain, a number show definite pallor. In 'panoptic' films some of the infested cells begin to show *very fine* stippling, which imparts to the cell a pinkish ground-glass appearance.

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\* The diameter of healthy red cells as seen in fixed and stained preparations of blood from *S. rhesus* varies from about 6.75 to 7.75 microns, the average being about 7.25 microns.

† The colour of the pigment seen appears to vary considerably with certain factors, such as the illumination used, the character and intensity of the stain, and the magnification employed.

When the parasite almost fills the red cell segmentation of the chromatin commences (18 to 21 hours). The early schizonts are solid, rounded bodies containing from 2 to 5 masses of chromatin (Plate V, figs. 27 and 28). Pigment tends to collect into several aggregations of coarse granules of a dark greenish brown or almost black colour. In Giemsa-stained films pallor of the infested cells is often marked at this stage, while in 'panoptic' films faint, irregular stippling of a rose pink colour is constantly seen. Unlike Schüffner's dots, this stippling is less uniform in size and it also stains less intensely.

With further division of the chromatin (21 to 24 hours), the parasite does not appear to increase in size, but the pigment granules tend to become aggregated into two or three dense clumps of a greenish or brownish black colour usually situated excentrically. Pallor of the infested cells is marked in films stained with Giemsa's stain, while in 'panoptic' films the infested cells show distinct stippling. The dots of stippling are frequently irregular in size and distribution, and in many cases the appearance presented is as if some of the very fine dots had coalesced to form larger ones (Plate V, figs. 29 and 30). Frequently the margin of the infested cell has an irregular 'fuzzy' appearance, while the interval between the cell margin and the parasite has a 'ghostly' aspect and is marked only by a few, irregular dots of stippling.

Sometimes the red cells infested by the segmenting forms, especially the more advanced ones, appear to be very slightly enlarged (about 8 microns), but the degree of enlargement is very slight in comparison with that seen in the corresponding forms of *P. vivax* and *P. inui* var. *cynomolgi*.

The mature schizont is usually rather smaller than a normal red cell, but may occasionally appear to be about the same size. The number of merozoites varies from 8 to 16\* (usually about 10) arranged either in the form of a rosette, or irregularly scattered. The pigment is aggregated into a single, very dark brown or black excentric mass (Plate V, fig. 31), or rarely, two such masses may be seen\*. In films stained with Giemsa's stain, the remains of the infested cell may be so pale as to be scarcely visible. In films stained by the 'panoptic' method, the infested red cell may be represented only by a number of red dots of stippling, or by a spinulose red rim encapsulating the parasite. In the latter instance the appearance presented is as if the cell had collapsed on the margin of the parasite.

*Sexual cycle.*—The mature gametocytes are spherical bodies almost completely filling the red cells, which are usually of approximately normal size. The protoplasm of the macrogametocyte stains deep blue. The chromatin, which is dense and compact, is usually situated peripherally and frequently shows a more deeply staining inner area (karyosome) surrounded by a less

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\*In no case have we been able to count more than 16 merozoites, where the possibility of double infection could be excluded with reasonable certainty. The presence of two large, discrete masses of pigment has, in some cases, suggested the probability of double infection (Plate V, fig. 30).

deeply staining areola. The pigment is scattered irregularly throughout the protoplasm and occurs as dark brown or almost black granules of moderate size (Plate V, figs. 32 and 33). In Giemsa-stained films the infested red cell is very pale, while in 'panoptic' films rather coarse red dots of stippling may be seen, or the parasite may be surrounded by a deep red spinulose capsule which often gives it a 'hedge-hog' appearance. The microgametocyte stains poorly and has frequently an indistinct pinkish purple colour. The chromatin is large and diffuse, and appears to merge into the protoplasm; it may occupy about 1/3rd of the entire parasite, may be situated centrally or peripherally, and may stain uniformly or show an inner, more intensely staining area (karyosome) (Plate V, figs. 34 and 35). The pigment granules resemble those seen in the macrogametocyte, but, on account of the relatively smaller amount of protoplasm, they appear to be more localized. The infested cells undergo alterations similar to those described for the macrogametocytes.

*Schizogony cycle.*—Twenty-four hours. Determined by successive blood examinations. Blood films were taken at intervals of 3 hours (and often more frequently), day and night, for from 24 to 48 hours from animals with established inoculation infections. From these slides it was found possible to follow the cycle of schizogony, and to estimate the approximate age of the parasites seen. In some instances the schizogony cycle was particularly well demonstrated, practically all the parasites seen at a given time being at the same stage of development.

Owing to the difficulties experienced in obtaining normal specimens of *S. irus*, and the high cost of these Malayan monkeys in India, we have not yet been able to study *P. knowlesi* infections so extensively in this species as has been done in *S. rhesus*. It has, however, been possible to obtain, by experimental inoculation, what we believe to be a pure strain of *P. knowlesi* in *S. irus*. With the exceptions of its milder pathogenic effects and the scantier parasites in the peripheral blood, no noteworthy differences have been detected between the morphology and schizogony cycle of *P. knowlesi* observed in *S. irus* and that described above for *S. rhesus*.

Similarly we have been unable to detect any appreciable differences in the cycle and morphology of this parasite in inoculation infections in *S. sinicus* and *Pygathrix schistaceus*.

*Pathogenicity.*—Variable. In natural infections of *S. irus* (mixed infections; 5 animals) no symptoms were apparent, and malarial infection was detectable only by blood examination. The occurrence of parasites in the peripheral blood was intermittent, the predominant species present at one time being *P. knowlesi* and at another *P. inui* var. *cynomolgi*. In three specimens of *S. irus* infected experimentally (mixed infections), the symptoms were slightly more severe than those seen in natural chronic infections—mild irregular fever occurred, followed later by spontaneous clinical cure: one of the animals died subsequently of pneumonia.

What appeared to be pure infections with *P. knowlesi* were obtained after inoculation of blood from naturally infected monkeys into the following species :—*S. irus*, *S. rhesus*, *S. sinicus* and *Pygathrix schistaceus*.

(a) *S. irus*.—In one monkey of this species infected experimentally with an apparently pure strain of *P. knowlesi* a mild infection ensued. No symptoms were observed and spontaneous recovery occurred. In the early stages a sharp rise of temperature was observed coincident with schizogony, i.e., daily.

(b) *S. rhesus* (15 animals).—The infection in this species of monkey was invariably fatal if untreated (7 animals); the animals showed high fever, great malaise, and acute pernicious symptoms, often with hæmoglobinuria; before death temperature becomes sub-normal. Hæmoglobinuria was seen in a total of 8 animals of which 5 had received no treatment. If treatment (*vide infra*) is begun on the first or second day after the appearance of parasites, the progress of the acute disease is usually arrested and a chronic infection, similar to that seen in *S. irus*, may ensue; sometimes, however, these chronic infections may be associated with severe fatal anæmia,\* although at the time of death parasites may be very scanty or undetectable in the peripheral blood. The post-mortem appearances of acute fatal infections are similar to those seen in pernicious attacks of human malaria; the capillaries of the internal organs and brain harbour innumerable parasites.

(c) *S. sinicus*.—Only 1 animal infected; this showed an acute attack. When parasite count reached 50,000 per c.mm. of blood, quinine, in doses of 2 grains daily, was given for 2 consecutive days; this animal then developed a mild chronic infection, without any apparent symptoms. It is possible that this species is not so extremely sensitive to the infection as is *S. rhesus*.

(d) *Pygathrix schistaceus*.—In the one animal injected, the infection was acute, accompanied by febrile and other symptoms. When the parasite count reached 135,000 per c.mm., quinine treatment was commenced and continued for 3 days. The infection became mild and chronic but the animal died 10 days later of pericarditis. Parasites were scanty at time of death.

*Treatment*.—No treatment was needed with *S. irus*, in which the infection recovers spontaneously. With *S. rhesus* good results have been obtained with quinine either alone or in combination with plasmoquine. If commenced on the 1st or 2nd day after the appearance of parasites in the peripheral blood, treatment is generally successful in changing an acute to a chronic infection, which may last at least one year. When, however, the disease is allowed to proceed unchecked for a longer period (i.e., parasite count rises above 100,000 per c.mm.) treatment is generally unsuccessful and, although the parasite count may be greatly reduced, death ensues.

The optimum dosage of quinine for small monkeys (about 3 kgr. weight) is about 2 grains daily, when given in solution by the mouth. This may be combined

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\* Some experiments, at present being carried out, suggest that stovarsol may be of value in these anæmic conditions.



with plasmoquine (0.0025 grm.) intramuscularly. Sufficient observations have not been made to make it possible to compare the efficacy of the different forms of treatment, taking into account the observed variations both in the reaction of the hosts and in the virulence of different strains of this parasite.

*Sporogony cycle in mosquitoes.\**

Franchini (1927) described a malaria parasite from the blood of a single specimen of *S. irus* (*M. cynomolgus*), the locality of origin of which was not known. He believed this *Plasmodium* to be different from *P. inui* and *P. inui* var. *cynomolgi*. From the description and the 19 uncoloured figures given, the characters of this parasite may be summarized as follows :—

*Appearances in stained preparations.†*

**Asexual cycle.** Mostly asexual forms seen. Youngest forms pyriform or rounded and situated at centre or periphery of red cell; chromatin relatively small and usually excentric; protoplasm pale blue, staining more intensely at periphery; vacuole small and may not be differentiated; pigment absent in youngest forms. With growth parasite increases in size and pigment appears in yellowish brown granules often peripherally situated. (Figures show pigment granules, resembling those in *P. vivax*; some figures suggest presence of 'accessory chromatin dot'.) Segmenting forms rarely observed; none seen with more than eight daughter nuclei; pigment in small granules. Infested red cells show characteristic changes; frequently diminished in size and often distorted (figures show some red cells rounded, shrunken and spinulose; others elongated and fimbriated, resembling those seen with *P. ovale*); hypercoloration seen in some red cells parasitized by smaller forms. No stippling seen.

**Sexual cycle.** Gametocytes rarely seen. Endoglobular macrogametocytes rounded and smaller than normal red cell; protoplasm stains deeply; pigment granules fine and numerous, scattered through protoplasm; chromatin peripheral and often falciform. Microgametocytes have same appearance but protoplasm stains less deeply, and yellowish brown pigment granules are more abundant. (Figures show 'sexual forms' as described, but some of these do not appear to us to be mature, and might possibly be immature asexual parasites.)

**Schizogony cycle.** Not determined.

**Pathogenicity.** No mention is made of illness in the infected monkey. The blood infection was apparently mild—at most one or two parasites in every microscopic field.

This parasite occurs in the same natural host (*S. irus*) as *P. knowlesi*, which it closely resembles in the marked and characteristic deformities shown by the infested cells in this host, in the yellowish brown character of the pigment, and in the possible occurrence of an 'accessory chromatin dot'. The absence of marked vacuolation and amœboidicity in the growing forms, and

\* While this paper was in the press, we have succeeded in demonstrating sporozoites in the salivary glands of *An. annularis* (*An. fuliginosus*) after feeding on a monkey infected with an apparently pure strain of *P. knowlesi*.

† Blood films stained with Giemsa's stain.

‡ It is very difficult in black and white figures to differentiate pigment granules from small masses of chromatin.

the solid, rounded appearance of these forms are other points of resemblance to *P. knowlesi* as seen by us in *S. irus* and *S. rhesus*. We, therefore, consider that this parasite should for the present be grouped as *P. knowlesi*.\*

Napier and Campbell (1932) discovered a *Plasmodium* in the blood of a specimen of *S. irus*†, purchased by them in Calcutta and said to have been imported from Singapore. This parasite is described as having many of the characters of *P. kochi*‡. These workers give no details of the morphology of this parasite, but found that, after parenteral inoculation, it produced a chronic infection in the same species of monkey, while in *S. rhesus* the infection was very severe. Such acute infections in the latter species of monkey usually killed the animal (11 out of 12 monkeys) and, in eight out of eleven animals which died, hæmoglobinuria was a marked feature of the terminal stages of the disease.§ The latter complication did not appear to have any connection with the administration of quinine. The virulence of the strain seemed to increase with repeated passage.

This strain of monkey malaria was the subject of further study by Knowles and Das Gupta (1932), who have given a detailed description and 12 coloured figures of the parasites as seen in the natural host, *S. irus*. For the reasons given in Appendix II, we believe that this description refers to a mixed infection of *P. inui* var. *cynomolgi* with *P. knowlesi*, and not to a pure infection with either parasite. We believe, however, that the minute description, given by these authors of the infection seen after passage into *S. rhesus*, refers entirely to *P. knowlesi*. From the description given and the twelve coloured figures which accompany it, the *Plasmodium* seen in *S. rhesus* has the following characters:—

*Appearances in stained preparations.||*

*Asexual cycle.* Young rings 1/3rd to 1/2 diameter red cell; resemble those of *P. falciparum* but usually larger and with more protoplasm, especially at side opposite chromatin; chromatin as prominent dot or blob, or sometimes as straight or curved bar, not infrequently within vacuole; chromatin frequently double (46 per cent), or even treble (3 per cent); rings frequently distorted, only small

\*Since the preparation of this paper for publication, Professor Franchini has very kindly sent us one of the original films made from the blood of the monkey from which he described this parasite. The parasites seen in this film appear to us to be indistinguishable from the forms of *P. knowlesi* seen about 6 to 9 hours after segmentation.

†The identification of this monkey as *Cercopithecus pygerythrus* in the original communication was later found to be incorrect, and has since been changed to *S. irus* (Knowles, 1932).

‡It seems probable that the parasite here referred to, is not *P. kochi* (Lav.), but the *P. kochi* of Gonder and Berenberg-Gossler (1908), which shows a very close resemblance to *P. inui* var. *cynomolgi*. We have classified the *Plasmodium* described by Gonder and Berenberg-Gossler as a variety of *P. inui* and have proposed the name *P. inui* var. *gonderi* for it (*vide supra*).

§Bouillies (1913) recorded the occurrence of hæmoglobinuria in a specimen of *Cercopithecus callitrichus* said to be infected with *P. inui*.

|| Blood films stained with Leishman's and Giemsa's stains combined.

proportion being small, compact and non-amoeboid (18 per cent); latter forms probably early sexual forms; accolé forms rarely seen in early phase of infection, but may be numerous in terminal phase; red cells unaltered at this stage—no enlargement, no stippling. Growing trophozoites non-amoeboid, or very slightly amoeboid, occupying 2/3rd or more of infested cell; very occasionally egg-like or band-like forms seen, resembling those of *P. malariae*; chromatin conspicuous blob-like mass usually lateral in position, or as band along one side of parasite; pigment in scattered, fine, brown-black grains; infested red cells show no evidence of enlargement or stippling, but may appear slightly smaller and definitely paler than normal. Growing schizonts occupy almost entire cell before segmentation of chromatin commences; protoplasm globular, non-amoeboid; 2 to 10 chromatin nuclei present; pigment at first scattered in brown-black granules, but later aggregated into dense black, excentric cluster; infested cells neither enlarged nor stippled, but occasionally appear smaller and paler than normal. Mature schizonts resemble those of *P. falciparum*, but number of merozoites is fewer—8 to 11 (usually 10) in grape-like cluster around dense mass black pigment; infested red cells neither enlarged nor stippled.

Heavy infections with this *Plasmodium* resemble acute infections with *P. falciparum*, on account of numerous cells showing multiple infection, abundant accolé forms, and early division of chromatin (ring forms).

*Sexual cycle.* Gametocytes intracellular; resemble those of *P. malariae*, but less deeply pigmented. *Macrogametocyte.* Globular, non-amoeboid body filling infested red cell; chromatin as large deeply staining mass situated peripherally and showing no evidence of less deeply staining outer zone; pigment in fine brown-black grains scattered through protoplasm; infested cell shows no enlargement and no stippling. *Microgametocyte.* Globular, non-amoeboid body, protoplasm of which stains poorly; chromatin as equatorial band more or less across centre of parasite—diffuse and stains poorly; pigment granules tend to be arranged around margin leaving centre relatively free from pigment; infested cells of normal size—may show pallor, but no enlargement or stippling seen.

*Schizogony cycle.* Not definitely determined, but requires further investigation. Certain findings 'may perhaps indicate a 24-hour cycle'.

*Pathogenicity.* Knowles and Das Gupta (1932) studied this subject in animals inoculated with the strain found by Napier and Campbell (1932). Their findings are summarized below:—

- (a) In *S. irus* (9 specimens) very mild infections, no symptoms observed; spontaneous clinical recovery the rule, sometimes followed by prolonged chronic infections. (We believe, however, as stated previously, that the most if not all, the infections in *S. irus* were mixed ones of *P. knowlesi* and *P. inui* var. *cynomolgi*.)
- (b)\* In *S. rhesus* (23 animals) very severe and acute infections, invariably fatal if untreated; animals show high fever becoming sub-normal before death, severe anemia; enlarged spleen and sometimes hæmoglobinuria; parasites may number 3½ millions per c.mm. before death.
- (c)\* In *S. sinicus* (*M. radiatus*) (4 animals) mild infections, usually with spontaneous recovery.
- (d)\* *Pygathrix entellus* (*Semnopithecus entellus*) (2 animals) seemed nearly as susceptible as *S. rhesus*.

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\*From the descriptions given of the parasites found in these inoculated infections, it seems to us that in most of them the predominant, if not the only, *Plasmodium* present was *P. knowlesi*.

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(e)\* *Hylobates hoolek* (1 animal) almost insusceptible.

(f)\* Of three human volunteers, one had an extremely severe infection, one a moderate infection with relapse and one a mild infection; all recovered spontaneously.† They think that virulence is probably enhanced by animal passage.

*Treatment.* In *S. irus*, infection readily cured by quinine, but tends to relapse if insufficient is given. In *S. rhesus*, many can sometimes be saved by quinine (1 grain daily for 4 to 5 days to monkeys weighing 2 to 3 kilogrammes)‡; smaller doses may induce chronic infection, if animal survives.

Colonel Knowles has very kindly supplied us with a specimen of *S. irus* and one of *S. rhesus* inoculated from his infected animals. This has enabled us to study the parasitic morphology described by Knowles and Das Gupta (1932) and to compare it with that of parasites found by us in natural infections of *S. irus*.

After a careful comparison we were unable to detect any morphological differences between the parasitic appearances seen by us in the *S. irus* sent by Colonel Knowles and those seen in *S. irus* infected in nature.\* We were also unable to discover any differences between the morphological and pathogenic characters of either of these strains when inoculated into *S. rhesus* or other monkeys.

For the reasons given in Appendix II, we are forced to believe that the natural infections seen by us in *S. irus* were mixed ones of *P. knowlesi* and *P. inui* var. *cynomolgi*, and that the original strain used by Knowles and Das Gupta (1932) was probably of a similar nature.† This Calcutta strain in *S. rhesus* has shown the characteristic morphology of *P. knowlesi* as described by us. In addition to the points described by Knowles and Das Gupta (1932) we have noted the peculiar deformities of the infested red cells, the presence of an 'accessory chromatin dot' and the occurrence of a 24-hour schizogony cycle.

In the course of our experimental work on immunity in malaria (Sinton and Mulligan, 1932a) we have had occasion to infect 44 specimens of *S. rhesus* with the Calcutta strain of *Plasmodium*. Of these, 16 received no treatment, and all either died, or were chloroformed when moribund to obtain material for the preparation of antigens. Hæmoglobinuria was observed in 9 out of 16 untreated animals. Twenty-eight infected monkeys received treatment (quinine; quinine combined with plasmoquine; plasmoquine; tebetren),

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\* From the descriptions given of the parasites found in these inoculated infections, it seems to us that in most of them the predominant, if not the only, *Plasmodium* present was *P. knowlesi*.

† This is apparently the first certain record of infection in man by a *Plasmodium* from the lower monkeys. Clark and Dunn (1931) have, however, reported some doubtful results with a monkey *Plasmodium* found in Panama. Attempts made by Berenberg-Gossler (1909) with *P. inui* var. *gonderi* failed. Blacklock and Adler (1922) were also unsuccessful in their efforts to transmit a *Plasmodium* of the chimpanzee to two human volunteers.

‡ Chopra, Das Gupta and Sen (1932) state that quinine treatment almost always fails when the number of infested red cells is 50 per cent or more in these infections.

but only seven which had received early treatment recovered. The remainder died either in the acute stage (including 7 which developed hæmoglobinuria), or later of severe anæmia.

Knowles and Das Gupta (1932) think that their parasite 'is probably identical with some species that has been (inadequately) described previously'. Such a view is apparently correct for, in our opinion, there is little doubt that these workers were dealing with two distinct parasites both of which had previously been described, although one (*P. knowlesi*) was previously an unnamed species.

*P. knowlesi* is, in our opinion, identical with the *Plasmodium* described by Knowles and Das Gupta (1932) in *S. rhesus*. The most important additional characters which we wish to emphasize from our own observations are (a) the definite 24-hour cycle of schizogony, (b) the presence of an 'accessory chromatin dot' in the young forms, and (c) the peculiar deformities of many of the infested red cells, many of which resemble those seen with *P. ovale*. Although we lack the confirmatory evidence as to the duration of the schizogony cycle, we believe that the *Plasmodium* described by Franchini (1927) is also identical with *P. knowlesi*.

#### *Discussion on P. knowlesi sp. n.*

The close similarity between the three different parasites, which have been included under this head, makes it appear almost certain that they belong to the same species. A careful study of blood slides from all these strains has confirmed this opinion.

The following characters are common to all three strains of parasite:—(a) natural host, *S. irus*; (b) infection mild in character in natural host; (c) segmenting forms present in peripheral blood; (d) morphology of parasite very similar in all respects; and (e) characteristic distortion of infested red cells.

The parasites of Knowles and Das Gupta (1932) and of the authors agree also in that (a) the natural hosts came from Malaya; (b) no noteworthy morphological differences could be detected at any stage of development; (c) a distinct 'accessory chromatin dot' is present in many of the young forms and merozoites; and (d) the infested red cells show no appreciable enlargement, and no stippling is seen with ordinary stains but can be demonstrated in cells parasitized by larger forms when special staining methods are employed.

In Franchini's parasite no stippling of the infested red cells was detected, but only Giemsa's stain was used. The occurrence of an 'accessory chromatin dot' is not reported, but its presence in the young forms is suggested by some of Franchini's figures.

The parasites, studied by Knowles and Das Gupta (1932) and by us, both show (a) a definite 24-hour cycle, and (b) very similar pathogenic effects on

experimentally infected monkeys. Neither of these characters has been determined for the *Plasmodium* described by Franchini (1927).

We do not think that these parasites can be considered to be identical with any of the previously named species of monkey *Plasmodium*. Our findings that the schizogony cycle in *P. knowlesi* is definitely a 24-hour one, and the occurrence of characteristic deformity of the infested red cells, are two characters which at once separate this parasite from all the other named Plasmodia of the lower monkeys of the Old World. The schizogony cycle of *P. semnopithecii* has not been determined, but it is clearly differentiated from *P. knowlesi* by its morphological characters, the absence of peculiar deformities of the infested cells, and of schizogony in the peripheral blood, by the genus of its natural host, and by its geographical distribution.

From the parasites of the '*P. kochi* group', *P. knowlesi* is distinguished by (a) its natural host and geographical distribution; (b) the presence of an 'accessory chromatin dot'; (c) segmenting forms common in the peripheral blood; (d) the occurrence of characteristic deformities of the infested red cells\*; (e) the ease with which it can be transmitted to other lower monkeys; and (f) the duration of the schizogony cycle.

With regard to the identity of this parasite, Franchini (1927) was unable to classify his parasite with any of the known species of monkey *Plasmodium*. He considered it to be different from both *P. inui* var. *cynomelgi* and *P. inui*, which had previously been described from *S. irus*. Similarly Knowles and Das Gupta (1932) refrained from identifying their parasite with any of the previously named species. We have been fortunate enough to be able to study two of these parasites in many infected animals, and to study a blood slide from the third one. We have also had access to most of the relevant literature on the subject in its original form. As the result of a critical study we consider, for the reasons given above, that there is no doubt that this is a previously-unnamed species of monkey *Plasmodium*. The name *P. knowlesi* was therefore proposed for this parasite (*vide* Sinton and Mulligan, 1932b) in honour of Lieut.-Col. R. Knowles, I.M.S., whose study of the morphology and pathogenicity of this parasite has contributed so much to our knowledge of monkey malaria.

The pathogenicity of this parasite is very interesting. The successful attempts of Knowles and Das Gupta (1932) to infect man with a *Plasmodium* from the lower monkeys, are the first on record. The fact that these patients recovered spontaneously suggests that they had possibly developed some immunity after previous attacks of human malaria, or that man is an unsuitable host.

The observations of various workers that plasmodial infections are found in nature more commonly in young specimens of *S. irus*, suggest that older animals have some immunity to the infection. The mild character of the

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\* On account of these peculiar oval and fimbriated cells we at one time thought that *P. knowlesi* might be identical with the human parasite, *P. ovale*, but when the schizogony cycle of the former proved to be 24 hours, this view could not be maintained.

infection in *S. irus* may be due to an acquired or a natural immunity, probably the former.

The large number of specimens of *S. rhesus* examined by us and by Knowles and Das Gupta (1932) from Northern India, without detecting any malarial infection, suggests that this species of monkey rarely, if ever, suffers from natural infections with malaria in this region. The absence of any opportunity for acquiring an immunity may account for the severity of the infections produced by *P. knowlesi* in this animal. On the other hand, this severity may be due to the introduction of a foreign parasite, i.e., a Malayan parasite into a Northern Indian host. The latter explanation might also apply to the results obtained with the langurs, *Py. entellus* and *Py. schistaceus*. The comparatively mild nature of the disease in the anthropoid ape (*H. hoolock*) and in man, seems to show that these higher Primates possess some natural immunity to the parasites of the lower monkeys. Such a view is supported by the failure of Berenberg-Gossler (1909) to infect man with *P. inui* var. *gonderi*, of Halberstadter and Prowazek (1907) to infect the orang-outang with *P. inui*, and of Leger and Bouilliez (1913) to infect the chimpanzee with the same species of parasite.

For full particulars of the morphology of *P. knowlesi* the reader is referred to the detailed description given by us above. The salient features of this parasite, so far as is known at present, may be summarized as follows :—

- (1) Natural infections in *S. irus*, probably from Malaya.
- (2) 'Accessory chromatin dot' present in young forms and merozoites.
- (3) Growing forms show no marked amœboidicity.
- (4) All stages of development found in the peripheral blood.
- (5) Infested red cells not appreciably enlarged, but often pale; stippling present with advanced growing forms and gametocytes, but only demonstrable with special stains; infested cells show characteristic distortion—oval, irregular, fimbriated, polyhedral or spinulose.
- (6) Schizogony cycle 24 hours.
- (7) Pathogenicity. Infections not severe in natural host; easily transmissible to some other species and genera of lower Oriental monkeys; symptoms in other experimentally infected monkeys variable, but very severe and usually fatal in *Silenus rhesus* and *Pygathrix entellus*; has been communicated to man by blood inoculation.
- (8) Sporogony cycle.\*

#### Discussion of the '*P. inui* group' of Plasmodia.

This group has been so fully discussed under the various species and varieties, that little remains to be said.

The natural hosts recorded for all these species have belonged either to the Oriental genus *Silenus* (*Macacus*) or to the allied African one, *Cercocebus*.

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\*Vide foot-note on p. 415.

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The parasites are all easily transmissible to other lower monkeys, in contradistinction to those of the '*P. kochi* group'.

The similarity between many points in the recorded morphology of the '*P. inui* group' and of *P. semnopithec*i cannot be overlooked. The latter parasite may eventually prove to be identical with *P. inui* or one of its varieties, but it is considered advisable for the present to retain it as a separate species pending more detailed information.

All the parasites of this group show a considerable morphological resemblance to each other and, except in the case of *P. knowlesi*, have a 48-hour schizogony cycle. It may possibly be advisable at a future date to separate the latter species from the '*P. inui* group', but for the present we have retained it along with the other Plasmodia of the lower Oriental monkeys.

The main differences between the '*P. inui* group' and the '*P. kochi* group' have already been summarized (p. 360). In Table IV are contrasted the chief diagnostic features recorded in the various parasites of the former group, as seen in their natural hosts.

TABLE IV.

*Chief differential characters of the Plasmodia of the 'P. inui group'.*

	<i>P. inui</i> Halb. and Prow, 1907.	<i>P. inui</i> var. <i>cynomolgi</i> Mayer, 1907.	<i>P. inui</i> var. <i>gondeni</i> var. n.	<i>P. knowlesi</i> sp. n.
Natural hosts.	<i>Silenus irus</i> . <i>S. nemestrinus</i> . <i>S. rhesus</i> . <i>S. lasiotis tcheliensis</i> .	<i>S. irus</i> .	<i>Cercocebus fuliginosus</i> . <i>Cercocebus aethiopicus</i> .	<i>S. irus</i> .
Regions from which recorded.	Borneo, Java, Sumatra, Tonkin.	Malaya, Java.	(Hamburg) Africa.	Malaya.
Duration of schizogony cycle.	48 hours.	48 hours.	48 hours.	24 hours.
Chromatin in young ring forms and merozoites.	No 'accessory dot' recorded.	'Accessory dot' present.	'Accessory dot' present.	'Accessory dot' present.
Trophozoites.	Little or no amoeboidicity; vacuolation often marked, even in segmenting forms.	Amoeboidicity may be pronounced; vacuole at first well developed but not usually seen in segmenting forms.	Active amoeboidicity in fresh; less evident in fixed smears; vacuole well developed at first but absent or small in segmenting forms.	Amoeboidicity slight or absent; vacuole small or absent in older trophozoites and segmenting forms.



TABLE IV—concl'd.

	<i>P. inui</i> Halb. and Prow., 1907.	<i>P. inui</i> var. <i>cynomolgi</i> Mayer, 1907.	<i>P. inui</i> var. <i>gonderi</i> var. n.	<i>P. knowlesi</i> sp. n.
Pigment in trophozoites.*	Golden-yellow to brown; appears early; very fine and abundant.	Golden-brown; appears later and is coarser and scantier than in <i>P. inui</i> .	Greenish-yellow brown; appears comparatively early; not very abundant.	Golden-brown to almost black; appears early; abundant.
Gametocytes.	About size of red cell. Pigment fine, scattered, yellowish brown, abundant.	Larger than red cell. Pigment not very abundant; darker and coarser than in <i>P. inui</i> .	Larger than red cell. Pigment coarse and abundant.	About size of normal red cell. Pigment relatively coarse, brown to black, abundant.
Infested red cells.	Never enlarged; may show hypercoloration. Stippling inconstant, sometimes, but rarely seen.	Enlarged and pale with larger parasites. Stippling usually present, and constant.	Enlargement and stippling with larger parasites; chiefly seen in experimental infections.	Not enlarged; pale with larger parasites; show characteristic distortion. Stippling only demonstrable with special stains.
Pathogenicity.	Variable, usually few or no symptoms. Easily inoculable to other lower monkeys, in which symptoms may be severe. Hæmoglobinuria recorded. Not inoculable to higher monkeys.	Variable; few or no symptoms. Easily inoculable to other lower monkeys, in which symptoms may be severe.	Mild. Easily inoculable to other lower monkeys of same species, but produces few symptoms. Failure to infect man.	Mild. Easily inoculable to other lower monkeys, in which symptoms may be very severe, often hæmoglobinuria. Has been transmitted to man and the gibbon.

\*From the various descriptions and figures it is often very difficult to determine the relative times at which pigment appears.

## OTHER PLASMODIA RECORDED AS NATURAL INFECTIONS IN ORIENTAL MONKEYS OF THE SUB-FAMILY CERCOPITHECINAE.

In addition to the natural malarial infections reported above, several authors have given other records of these in the genus *Silenus*. We have been unable to classify some of these parasites.

### *Plasmodium semnopithecii* Knowles, 1919.

*P. semnopithecii*. Knowles (1919).

*P. semnopithecii*. Wenyon (1926).

*Plasmodium* sp. Chimento (1922).

This species has been fully described earlier in this paper, when dealing with the parasites found in the sub-family COLOBINÆ.

Chimento (1922), working in Italy, discovered a *Plasmodium* in the blood of *S. irus* (*M. cynomolgus*). The description given of this parasite corresponds

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more closely to *P. semnopithecii* than to any of the other monkey Plasmodia (*vide supra*).

Wenyon (1926) also records, without any description, the occurrence of *P. semnopithecii* in a specimen of *Presbytes pileatus* (? *Silenus pileatus*) from Assam, examined at the London Zoo.

### **Plasmodium kochi** (Laveran), 1899.

Castellani and Chalmers (1913) state that illness and death may occur among the monkeys of Ceylon, due to a malarial parasite which they refer to as *P. kochi*. No description of this parasite is given and this report, the first record of this African *Plasmodium* as a natural infection of Oriental monkeys, will require confirmation.

### **Plasmodium** sp. Green, 1932.

Green (1932) also reported the occurrence of malarial parasites in the blood of the 'Berok' or pig-tailed macaque (*S. nemestrinus*)\*, caught in the Malayan jungle a few miles from Kuala Lumpur. This parasite 'was very similar in some respects to those found in *S. irus* (*vide P. inui* var. *cynomolgi*), but differs in others in being more sluggish in movement, retaining the ring form until this almost fills the corpuscle, and producing dense greenish pigment which tends to obscure the protoplasm shortly after the ring forms start to grow. The chromatin mass of the growing ring forms is relatively very large'. It is impossible to classify this parasite definitely from the data given. The description has several points of resemblance to both *P. inui* (sens. restr.) and *P. knowlesi*.

## GENERAL DISCUSSION OF THE MALARIAL PARASITES OF THE LOWER MONKEYS OF THE OLD WORLD.

A provisional classification of the various Plasmodia described from the Lower Primates of the Old World has been given in this paper. A critical study of the relevant literature has shown that it is difficult or impossible to reconcile the considerable morphological differences recorded between various parasites which have been grouped in a single species. We have, therefore, created several new varieties of existing species in which future workers may be able to 'pigeon-hole' their findings until more definite information is available. It is hoped that by this means any further increase of the present confusion will be avoided.

We feel sure that our action in creating these new varieties will arouse considerable criticism. Nevertheless this course has been deliberately followed, because it seemed to be an essential preliminary step towards the ultimate elucidation of the problem. We are fully aware that in this classification morphological and other differences have been used which may eventually prove more apparent than real. Such differences may be dependent either upon the inadequacy of, or inaccuracies in, the original description, or upon

variations due to the species or genus of the natural host.\* When such doubtful points are cleared up, the new varieties can then be sunk in the parent species or elevated to specific rank if found necessary.

In carrying out this work considerable difficulty has been experienced in interpreting and evaluating the descriptions and figures given of certain characters. A discussion of such difficulties in some detail may be helpful to future workers.

(a) *Stippling in infested red cells*.—In human malarial infections, the demonstration of stippling in infested red cell is well known to depend, in many instances, upon the nature of the stains and reagents used. This has also been our experience with monkey *Plasmodia*, in which more consistent results were obtained with the panoptic methods, although, with attention to technique, Giemsa's and Leishman's stains usually revealed this phenomenon. Stippling in human malarial infections may be well marked at one part of a blood slide, while less so in other places on the same slide. Many workers have failed to demonstrate this character with several strains of monkey *Plasmodia*, although they had made special efforts to do so and had been able to find it with other species of *Plasmodium*, either human or simian, stained at the same time with the same technique. Under such circumstances the recorded absence of stippling cannot be lightly dismissed as due to the technique used.

It may be, however, that with some species of *Plasmodium* this character is sometimes evident and sometimes not, as in the case of Maurer's dots in infections with *P. falciparum* and Ziemann's dots with *P. malariae*. Our experience has been, however, that in infections with *P. vivax* this character is comparatively easy to demonstrate, more especially with the older forms of parasite. *P. inui* var. *cynomolgi* very closely resembles the latter parasite and, like it, produces a stippling which is easy to stain. Under these conditions, the presence or absence of stippling with the larger forms of this group of parasites must be taken as a point of great diagnostic importance, if special efforts have been made to demonstrate it.†

The presence or absence of distortion among the infested cells should also be recorded.

(b) *Pigment*.—Considerable difficulty has been experienced in deciding the exact nature of the pigment characters given in many of the descriptions and shown in the figures studied. It is not easy to depict accurately, either the colour or size of pigment granules, and in descriptions such terms as 'coarse'

\*In the light of recent work (*vide* Appendix II), the evidence in support of such variations of morphology in hosts of different species requires further confirmation.

†Some of the discrepancies in the reports of stippling may be due to the presence of an undetected mixed infection, occurring either naturally or picked up during the sub-passages of the infection.

and 'fine' are only relative. A comparison of different figures with their descriptions and with each other, often shows that pigment, which from the figures would appear to be of a very similar character, may be described by one author as 'fine' and by another as 'coarse'.

The period of the cycle at which pigment first becomes evident, appears to be of importance, but in many descriptions it has been impossible to find any definite information on this point. In recording the time when pigment first appears, it is not sufficient to state that it appears 'early' or 'late', because these terms would have very different meanings in a parasite with a 24-hour cycle as compared with one having a 48-hour cycle. The time of appearance may be noted as occurring in rings or parasites of such and such a size, or better still this description might be augmented by a statement as to the approximate age of the parasite at this time.

(c) *Amœboidicity*.—The degree of amœboidicity shown by parasites at different stages of development is another important diagnostic feature. Here again the opinions of different authors as to what constitutes evidence of this morphological character in stained specimens appear very diverse, if one compares the different figures and descriptions with each other.

(d) *Morphological variations*.—Knowles and Das Gupta (1932) have recorded marked variations in the morphology of what they considered to be the same species of parasite, when studied in simian hosts of different genera and species. Similar variations from quartan to tertian-like characters have been reported by Taliaferro (1932), in the malarial parasites found in monkeys in Panama, when observed in hosts of different genera.

In our earlier experiments with natural infections in *S. irus*, we observed the same apparent changes which Knowles and Das Gupta (1932) recorded. While keeping in mind the possibility that a mixed infection might be the cause of these variations, we were unable to prove it. However, more recently we have been able to obtain evidence (Appendix II) which appears to indicate clearly that such mixed infections are the explanation of most, and probably all, of the changes seen by us in our experiments.

While we have no evidence that such variations may not occur with other species of Plasmodia, we consider that the possibility of mixed infections as the only cause of such apparent morphological differences, requires further and very careful study. It has been our experience that unsuspected infections of this nature are much more common in human malaria than is generally believed. One species of parasite may predominate at one stage of infection, while the other species may be most common at a different period. In transmission experiments the recipient species or genus may be more susceptible to one of these species of parasite than to the other. Under these circumstances it is impossible to predict which species will be most evident in the new infection.

When such apparent variations in morphology are found in what appears to be a pure infection, it is essential that the character of the parasite should

be carefully described in each species of host in which it shows such variations, as was done by Knowles and Das Gupta (1932).

(e) *Pathogenicity*.—It has been suggested by some workers that pathogenicity may prove a useful differential character. This may eventually be found to be the case. It must be remembered, however, as pointed out by Blanchard and Langeron (1912), by Leger and Bouilliez (1913), by Seidelin and Connal (1914), and as has been discussed earlier in this review, that the pathogenic effects of experimental infections may depend upon whether the animal used had previously developed an immunity to the disease. The diversity of results, recorded with apparently the same parasite in the same species of host, may depend upon whether the animal has had a previous infection with the same strain of parasite and thus acquired some immunity.\* Flu (1908) reported that previous infection made later ones much milder.

Some workers have recorded the sudden awakening of latent and unsuspected infections in monkeys which were apparently in normal health. These infections were lighted up in some instances by protein shock (Knowles, 1919) or by traumatism (Sergeant, 1908).† In some areas the reported incidence of detectable malaria among monkeys, especially in young ones, seems high. Among 11 young specimens of *S. irus* examined by us, four were found infected. These findings suggest that the wide variations in immunity recorded may, in some instances at least, be dependent upon an immunity acquired as the result of an infection in early life. We have already remarked upon the severity of inoculated infections in *S. rhesus*, a species in which there is some evidence to show that the natural incidence of malaria, in Northern India at least, is very slight or possibly absent.‡

### SUMMARY.

Full abstracts have been given of the chief descriptions of the malarial parasites recorded from the different species of lower monkey found in the Old World.

An attempt has been made to clarify the literature on this subject and to review it critically.

As a result of this work it has been found possible to divide these parasites into definite groups, based on their morphology, their pathogenicity and their natural hosts.

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\* Some recent experiments in these laboratories go to show that, while one strain of *P. knowlesi* will produce considerable immunity against superinfection with the same strain, this is not always the case with a different strain.

† We have found that the intravenous injection of a few c.cs. of human blood into a monkey with a latent infection, causes the rapid reappearance of parasites in the peripheral blood.

‡ Although we have examined between 200 and 300 monkeys of this species, we have never been able to find a natural infection. Knowles and Das Gupta (1932) record a similar failure to find such infections in the same animal.

The following is the tentative classification adopted :—

- (i) *Plasmodium kochi* (Lav.), 1899, sens. restr.  
     'Malariaähnlichen Blutparasiten bei Affen'. Koch (1898);  
     Kossel (1899).  
     *Haemamæba kochi*. Laveran (1899).  
     *P. kochi*. Sergent (1908).  
     *P. cercopithecii*. Theiler (1930).
- (a) *Plasmodium kochi* var. *bouilliezi* Leger, 1922.  
     *P. bouilliezi*. Leger (1922).  
     *P. kochi*. Anderson and Cowdry (1928).
- (b) *Plasmodium kochi* var. *joyeuxi* Leger, 1928.  
     *P. joyeuxi*. Leger (1928).  
     *Plasmodium* resembling *P. kochi*. Martoglio *et al.* (1910).  
     *P. kochi*. Joyeux (1913); Bouilliez (1916).
- (c) *Plasmodium kochi* var. *macfiei* var. n. Sinton and Mulligan, 1932.  
     '*P. inui*'. Macfie (1928).  
     *Plasmodium* sp. Seidelin and Connal (1914); Connal and Coghill (1916).  
     *Plasmodium* sp. Grigorieva (1929).  
     *P. kochi*. Theiler (1930).  
     *P. kochi* var. *macfiei*. Sinton and Mulligan (1932b).
- (ii) *Plasmodium inui* Halberstadter and Prowazek, 1907 (sens. restr.).  
     *P. inui*. Mathis and Leger (1911).  
     *P. inui*. Leger and Bouilliez (1912; 1913); Bouilliez (1913).
- (a) *Plasmodium inui* var. *cynomolgi* Mayer, 1907.  
     *P. cynomolgi*. Mayer (1907; 1908); Flu (1908).  
     *P. cynomolgi*. Blanchard and Langeron (1912; 1913).  
     *P. cynomolgi* (?). Donovan (1920).  
     *P. inui* (?). Green (1931; 1932); Kingsbury (1931).  
     *P. kochi* (?) (pro parte). Napier and Campbell (1932).  
     *Plasmodium* sp. (pro parte). Knowles and Das Gupta (1932).  
     *Plasmodium* sp. (pro parte). Sinton and Mulligan (1932).  
     *P. inui* var. *cynomolgi*. Sinton and Mulligan.
- (b) *Plasmodium inui* var. *gonderi* var. n. Sinton and Mulligan, 1932.  
     *P. kochi*. Gonder and Berenberg-Gossler (1908); Berenberg-Gossler (1909); Gonder and Rodenwaldt (1910).  
     *P. kochi*. Grigorieva (1929).  
     *P. inui* var. *gonderi*. Sinton and Mulligan (1932b).
- (iii) *Plasmodium knowlesi* sp. n. Sinton and Mulligan, 1932.  
     *Plasmodium* sp. Franchini (1927).  
     *P. kochi* (?) (pro parte). Napier and Campbell (1932).  
     *Plasmodium* sp. (pro parte). Knowles and Das Gupta (1932).  
     *Plasmodium* sp. (pro parte). Sinton and Mulligan (1932).  
     *P. knowlesi*. Sinton and Mulligan (1932b).

(iv) *Plasmodium semnopithecii* Knowles, 1919.

*P. semnopithecii*. Knowles (1919).

*Plasmodium* sp. Chimosso (1922).

*P. semnopithecii*. Wenyon (1926).

It is recognized that the grouping suggested here may require considerable alteration or modification as more detailed knowledge of these Plasmodia is accumulated. It is hoped, however, that the data and suggestions given above may be the first step towards a more accurate and scientific classification of this important genus of similar parasite.

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## APPENDIX I.

## (A). RECORDS OF PLASMODIAL INFECTIONS IN THE LOWER AFRICAN MONKEYS.\*

## (1) Natural infections.

Genus *Colobus*.

<i>C. rufimitratus</i>	<i>Plasmodium</i> sp.	Belgian Congo.	Strong and Shattuck (1930); Theiler (1930).
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Genus *Cercopithecus*.

<i>C. albogularis</i>	<i>P. kochi</i>	(Paris)	Sergent (1908).
<i>C. callitrichus</i>	<i>P. kochi</i> ( <i>P. kochi</i> var. <i>joyeuxi</i> )	French Guinea	Joyeux (1913).
<i>C. callitrichus</i>	<i>P. kochi</i> ( <i>P. kochi</i> var. <i>joyeuxi</i> )	Central Africa	Bouilliez (1916).
<i>C. callitrichus</i>	<i>P. joyeuxi</i> ( <i>P. kochi</i> var. <i>joyeuxi</i> )	French Guinea	Leger (1928).
<i>C. callitrichus</i>	<i>P. kochi</i> (?)	(Paris)	Leger and Bouilliez (1913).
<i>C. campbelli</i>	<i>P. bouilliezi</i> ( <i>P. kochi</i> var. <i>bouilliezi</i> )	French Guinea	Leger (1922; 1928).
<i>C. cephus</i>	<i>P. kochi</i> (?)	Congo	Ringebach (1914).
<i>C. diana</i>	<i>P. kochi</i> ( <i>P. kochi</i> var. <i>macflei</i> )	Liberia	Strong and Shattuck (1930); Theiler (1930).
<i>C. mona.</i>	<i>Plasmodium</i> sp.	Nigeria	Seidelin and Connal (1914); Connal and Coghill (1916).
<i>C. nictitans</i>	<i>P. cercopitheci</i> ( <i>P. kochi</i> )	Liberia	Strong and Shattuck (1930); Theiler (1930).
<i>C. pygerythrus</i> ( <i>C. erythrotis</i> )	<i>P. kochi</i> (?)	Gold Coast (London Zoo)	Wenyon (1926).
<i>C. pygerythrus</i>	<i>P. kochi</i> (?)	Rhodesia	Kinghorn and Yorke (1912).

\*The name of the monkey given in brackets is that used in the original record. The name of the *Plasmodium* given in brackets is the name now suggested in the classification used in this paper. When the locality is given in brackets, it indicates the place where the observation was made, as the region of origin of the host has not been mentioned.



( <i>C. pygerythrus</i> )* ? <i>P. kochi</i> ( <i>P. knowlesi</i> and <i>P. inui</i> var. <i>cynomolgi</i> ).	? Malaya (Calcutta)	Napier and Campbell (1932).
( <i>C. pygerythrus</i> )* <i>Plasmodium</i> sp. ( <i>P. knowlesi</i> and <i>P. inui</i> var. <i>cynomolgi</i> ).	? Malaya (Calcutta)	Knowles and Das Gupta (1932).
<i>C. sabaeus</i> <i>Plasmodium</i> sp. ( <i>P. kochi</i> )	East Africa	Koch (1898); Kossel (1899).
<i>C. sabaeus</i> <i>Plasmodium</i> sp. (?)	West Africa	Ziemann (1900).
<i>C. sabaeus</i> <i>P. kochi</i> (? <i>P. inui</i> var. <i>gonderi</i> )	(Hamburg)	Berenberg-Gossler (1909).
<i>C. sabaeus</i> <i>P. kochi</i> (?)	Sierra Leone	Plimmer (1912).
<i>C. sabaeus</i> ? <i>P. kochi</i> ( <i>P. kochi</i> var. <i>joyeuzi</i> )	Ethiopia	Martoglio <i>et al.</i> (1910).
<i>Cercopithecus</i> sp. <i>P. kochi</i> (?)	Africa	Koch (1906).†
<i>Cercopithecus</i> sp. <i>P. kochi</i> (?)	Uganda	Bruce and Nabarro (1903); Gray and Tulloch (1907); Ross (1907).
<i>Cercopithecus</i> sp. <i>P. kochi</i> (?)	Congo	Dutton, Todd and Tobey (1906).

Genus *Cercocebus*.

<i>C. aethiopicus</i> <i>P. kochi</i> (?)	(London Zoo)	Plimmer (1916).
<i>C. aethiopicus</i> <i>P. kochi</i> ( <i>P. inui</i> var. <i>gonderi</i> )	(Moscow Zoo)	Grigorieva (1929).
<i>C. fuliginosus</i> <i>P. kochi</i> ( <i>P. inui</i> var. <i>gonderi</i> )	(Hamburg Zoo)	Gonder and Berenberg-Gossler (1908); Berenberg-Gossler (1909).
<i>Cercocebus</i> sp. <i>P. kochi</i> (?)	Africa	Koch (1906).†

Genus *Erythrocebus*.

<i>E. patas</i> <i>P. kochi</i> (?)	French Guinea	Quoted by Stiles and Nolan (1929).‡
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Genus *Papio* (*Cynocephalus*).

<i>P. babuinus</i> <i>Plasmodium</i> sp.	East Africa	Koch (1898); Kossel (1899).
<i>P. pruinus</i> <i>Plasmodium</i> sp.	Nyasaland	Lamborn (1929).

\* The identification of this monkey as *C. pygerythrus* in the original communication has since been changed to *Silenus irus*, an Oriental monkey (Knowles, 1932).

† Quoted by Wenyon (1926).

‡ We have been unable to trace the origin of this record. The only records we can find regarding monkey malaria in this species are the experimental inoculation of *P. inui* by Leger and Bouilliez (1912; 1913), and the unsuccessful attempts of Bouilliez (1916) in Central Africa and of Leger (1928) in French Guinea, to infect this monkey with *P. kochi* var. *joyeuzi*.

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<i>P. sphinx</i>	<i>P. kochi</i> (?)	French Guinea	Leger (1928).
<i>P. sphinx</i>	<i>Plasmodium</i> sp. ( <i>P. kochi</i> var. <i>macfiei</i> )	Nigeria	Seidelin and Connal (1914); Connal and Coghill (1916).
<i>P. sphinx</i>	? <i>P. inui</i> ( <i>P. kochi</i> var. <i>macfiei</i> )	Gold Coast	Macfie (1928).
<i>P. sphinx</i>	? <i>P. inui</i> * ( <i>P. kochi</i> var. <i>macfiei</i> ?)	(Moscow Zoo)	Grigorieva (1929).
<i>Papio</i> sp. ( <i>Cynocephalus</i> sp.)	<i>Plasmodium</i> sp. ( <i>P. kochi</i> ?)	East Africa	Kossel (1899).

### Genus *Callithrix*.†

<i>C. personata</i> (?)	<i>P. kochi</i> ( <i>P. kochi</i> var. <i>bouilliezi</i> )	Senegal	Anderson and Cowdry (1928); Cowdry and Coghill (1928).
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### Genus and Species Unidentified.

sp.	<i>Plasmodium</i> sp.	(Berlin)	Kossel (1899).
sp.	<i>Plasmodium</i> sp.‡	Uganda	Duke (1921).

\* Grigorieva (1929) recorded this parasite in a young specimen of *P. sphinx*. Since this paper went to press, we have to thank Dr. Asheshov and Dr. Tscherbakoff for a translation of this Russian article. The description given would appear to be:—*Asexual cycle*. No rings seen; smallest asexual forms observed almost filled red cell. *Sexual cycle*. Macrogametocytes slightly larger than red cell; greenish-blue protoplasm, with fine, greenish-brown pigment evenly distributed; chromatin compact mass surrounded by light pink zone, usually peripherally situated, seldom central. Microgametocytes with light pinkish-yellow protoplasm, reticulate and with fine, light brown, evenly distributed pigment; nucleus intense pink with loose ball of chromatin. *Schizogony cycle*. Not determined. *Pathogenicity*. Latent infection probably lighted up by adrenalin; no clinical symptoms but marked anæmia. Grigorieva (1929) thinks this parasite similar to that of Seidelin and Connal (1914) and Macfie (1928), i.e., *P. kochi* var. *macfiei* from the same natural host. This seems probable, but the evidence appears insufficient on which to make any definite statement.

† These monkeys were said to come from Senegal. This genus are American monkeys of the family *CERAMÆ* and are not indigenous to Africa. If the identification is correct, this is the first record of the natural occurrence of *P. kochi*, an African *Plasmodium*, in an American species of monkey.

‡ Duke (1921) reports that malaria is very common among laboratory monkeys in Uganda, but does not mention the species of animal. Recently we have obtained the original paper and the description given of the *Plasmodium* is very scanty:—In some ways resembling the benign tertian type; with a great deal of pigment; no rosettes were seen in the peripheral blood. 'Their appearance suggests the parasite described by Knowles (1919) in that forms apparently free in the plasma are common. No crescents have been observed. The appearances ascribed by Knowles to chromolysis, plasmolysis, etc., are common'. 'While some of the monkeys bring their infection with them, others apparently become infected in the laboratory'. 'The infection, even though massive, does not seem seriously to inconvenience them'. We have not found it possible to identify this parasite from the data given; it would, however, appear to belong to the *P. kochi* group.

**(2) Experimental infections.**Genus *Cercopithecus*.

<i>C. callitrichus</i>	<i>P. inui</i>	Bouilliez (1913); Leger and Bouilliez (1912; 1913).
<i>C. cephus</i>	<i>P. inui</i>	Bouilliez (1913); Leger and Bouilliez (1912; 1913).
( <i>C. pygerythrus</i> )*	? <i>P. kochi</i> ( <i>P. knowlesi</i> and <i>P. inui</i> var. <i>cynomolgi</i> ).	Napier and Campbell (1932).
( <i>C. pygerythrus</i> )*	<i>Plasmodium</i> sp. ( <i>P. knowlesi</i> and <i>P. inui</i> var. <i>cynomolgi</i> ).	Knowles and Das Gupta (1932).
<i>Cercopithecus</i> sp.	<i>P. cynomolgi</i> ( <i>P. inui</i> var. <i>cynomolgi</i> )	Mayer (1908).

Genus *Cercocebus*.

<i>C. fuliginosus</i>	<i>P. kochi</i> ( <i>P. inui</i> var. <i>gonderi</i> )	Gonder and Berenberg-Gossler (1908); Berenberg-Gossler (1909); Gonder und Rodenwaldt (1910).
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Genus *Erythrocebus*.

<i>E. patas</i>	<i>P. inui</i>	Leger and Bouilliez (1912; 1913).
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Genus *Papio* (*Cynocephalus*).

<i>P. anubis</i>	<i>P. inui</i>	Leger and Bouilliez (1912; 1913).
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Genus *Callithrix*.†

? <i>C. personata</i>	<i>P. kochi</i> ( <i>P. kochi</i> var. <i>bouilliezi</i> )	Anderson and Cowdry (1928); Cowdry and Cowell (1928).
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**(3) Unsuccessful inoculation experiments.**Genus *Cercopithecus*.

<i>C. callitrichus</i>	<i>P. kochi</i> ( <i>P. kochi</i> var. <i>joyeuxi</i> )	Joyeux (1913); Bouilliez (1916).
<i>C. callitrichus</i>	<i>P. joyeuxi</i> ( <i>P. kochi</i> var. <i>joyeuxi</i> )	Leger (1928).
<i>C. mona</i>	? <i>P. inui</i> ( <i>P. kochi</i> var. <i>macfiei</i> )	Macfie (1928).

Genus *Cercocebus*.

<i>C. fuliginosus</i>	<i>P. inui</i>	Leger and Bouilliez (1912; 1913).
<i>C. lunulatus</i>	? <i>P. inui</i> ( <i>P. kochi</i> var. <i>macfiei</i> )	Macfie (1928).

\* The identification of this monkey as *C. pygerythrus* in the original communication has since been corrected to *Silenus irus* (*Macacus cynomolgus*), an Oriental monkey.

† These specimens were said to have come from Senegal. The genus, *Callithrix*, however, belongs to the American family CEBIDÆ, which is not indigenous to Africa.

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### Genus *Erythrocebus*.

<i>E. patas</i>	<i>P. kochi</i> ( <i>P. kochi</i> var. <i>joyeuxi</i> )	Bouillies (1916).
<i>E. patas</i>	<i>P. joyeuxi</i> ( <i>P. kochi</i> var. <i>joyeuxi</i> )	Leger (1928).
<i>E. patas</i>	? <i>P. inui</i> ( <i>P. kochi</i> var. <i>macfiei</i> )	Macfie (1928).

### Genus *Papio* (*Cynocephalus*).

<i>Papio</i> sp.	<i>P. joyeuxi</i> ( <i>P. kochi</i> var. <i>joyeuxi</i> )	Leger (1928).
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### Genus and Species Unidentified.

sp.	<i>Plasmodium</i> sp. ( <i>P. kochi</i> )	Kossel (1899).
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## (B). RECORDS OF PLASMODIAL INFECTIONS IN LOWER ORIENTAL MONKEYS.\*

### (1) Natural infections.

#### Genus *Pygathrix* (*Semnopithecus*; *Presbytis*).

<i>P. entellus</i>	<i>P. semnopithecus</i>	Assam	Knowles (1919).
<i>Presbytis pileatus</i> (sic!)	<i>P. semnopithecus</i> †	Assam	Wenyon (1926).

#### Genus *Silenus* (*Macacus*).

<i>S. irus</i> ( <i>M. cynomolgus</i> )	<i>P. inui</i>	Java	Halberstadter and Prowazek (1907).
<i>S. irus</i> ( <i>M. cynomolgus</i> )	<i>P. cynomolgi</i> ( <i>P. inui</i> var. <i>cynomolgi</i> )	Java	Mayer (1907; 1908).
<i>S. irus</i> ( <i>M. cynomolgus</i> )	<i>P. cynomolgi</i> ( <i>P. inui</i> var. <i>cynomolgi</i> )	(Paris)	Blanchard and Langeron (1912).
<i>S. irus</i> ( <i>M. cynomolgus</i> )	<i>P. inui</i>	(Paris)	Leger and Bouillies (1912; 1913).
<i>S. irus</i> ( <i>M. cynomolgus</i> )	<i>Plasmodium</i> sp. ( <i>P. knowlesi</i> )	(Italy)	Franchini (1927).
<i>S. irus</i>	? <i>P. inui</i> ( <i>P. inui</i> var. <i>cynomolgi</i> )	Malaya	Kingsbury (1931); Green (1931; 1932).
<i>S. irus mordax</i>	? <i>P. inui</i> ( <i>P. inui</i> var. <i>cynomolgi</i> )	? Java	Green (1932).

\*Vide foot-note to Appendix I (A).

†Vide foot-note under *Silenus pileatus*.

<i>S. irus</i> ( <i>C. pygerythrus</i> )*	? <i>P. kochi</i> ( <i>P. knowlesi</i> and <i>P. inui</i> var. <i>cynomolgi</i> ).	? Singapore (Calcutta)	Napier and Campbell (1932).
<i>S. irus</i> ( <i>C. pygerythrus</i> )*	<i>Plasmodium</i> sp. ( <i>P. knowlesi</i> and <i>P. inui</i> var. <i>cynomolgi</i> ).	? Singapore (Calcutta)	Knowles and Das Gupta (1932).
<i>S. irus</i> ( <i>S. pileatus</i> )†	<i>Plasmodium</i> sp. ( <i>P. knowlesi</i> and <i>P. inui</i> var. <i>cynomolgi</i> )	? Singapore	Sinton and Mulligan (1932a).
<i>S. irus</i>	<i>P. kochi</i> (?)	?	Quoted by Stiles and Nolan (1929) ‡
<i>S. lasiotis</i> <i>tcheliensis</i> .	<i>P. inui</i>	Tonkin	Mathis and Leger (1911).
<i>S. nemestrinus</i>	<i>P. inui</i>	Borneo and Sumatra.	Halberstadter and Prowasek (1907).
<i>S. nemestrinus</i>	<i>Plasmodium</i> sp. (?)	Malaya	Green (1932).
<i>S. pileatus</i> §	<i>P. semnopithecii</i>	(London Zoo)	Quoted by Stiles and Nolan (1929) §
<i>S. rhesus</i>	<i>P. inui</i>	Tonkin	Mathis and Leger (1911).
<i>S. rhesus</i>	<i>Plasmodium</i> sp.	Uganda	Bruce and Nabarro (1903).
<i>S. rhesus</i>	<i>Plasmodium</i> sp. ( <i>P. semnopithecii</i> )	(Italy)	Chimisso (1922).
<i>S. sinicus</i>	<i>Plasmodium</i> sp. (? <i>P. inui</i> var. <i>cyno-</i> <i>molgi</i> ).	South India	Donovan (1920).
? <i>Silenus</i> sp.	<i>P. kochi</i> (?)	Ceylon	Castellani and Chalmers (1913).
<i>Silenus</i> sp.	<i>Plasmodium</i> sp.	(Paris)	Leger and Bouilliez (1913).

## (2) Experimental infections.

Genus *Pygathrix* (*Semnopithecus*; *Presbytis*).

<i>P. entellus</i>	<i>Plasmodium</i> sp. ( <i>P. knowlesi</i> )	Knowles and Das Gupta (1932).
<i>P. schistaceus</i>	<i>P. knowlesi</i>	Sinton and Mulligan.

\* The diagnosis of this species of monkey as *C. pygerythrus* in the original communication has since been corrected to *S. irus*.

† The diagnosis of this species of monkey as *S. pileatus* in the original communication has since been corrected to *S. irus*.

‡ We have been unable to trace the origin of this record.

§ We have been unable to trace the origin of this record, but it appears to be that given by Wenyon (1926) (p. 1362) to the occurrence of *P. semnopithecii* in a specimen of *Presbytis pileatus* (sic!) from Assam, examined at the London Zoo. The name *Presbytis* is given by Stiles and Nolan (1929) as a synonym for the genus *Pygathrix* (*Semnopithecus*), the langurs, and not for *Silenus*, the macaques. This *Plasmodium* was apparently found in an Assamese monkey, but we have no information as to whether the names *Presbytis pileatus* and *Silenus pileatus* are synonymous. They probably are so, as we have not been able to trace a species of this name in the former genus, while the latter monkey has been recorded from Assam.

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### Genus *Silenus* (*Macacus*).

<i>S. irus</i> ( <i>M. cynomolgus</i> )	<i>P. cynomolgi</i> ( <i>P. inui</i> var. <i>cynomolgi</i> )	Mayer (1908); Flu (1908).
<i>S. irus</i> ( <i>M. cynomolgus</i> )	<i>P. inui</i>	Leger and Bouilliez (1912; 1913); Bouilliez (1913).
<i>S. irus</i> ( <i>M. cynomolgus</i> )	<i>P. cynomolgi</i> ( <i>P. inui</i> var. <i>cynomolgi</i> )	Blanchard and Langeron (1912; 1913).
<i>S. irus</i>	? <i>P. inui</i> ( <i>P. inui</i> var. <i>cynomolgi</i> )	Green (1932).
<i>S. irus</i> ( <i>C. pygerythrus</i> )	? <i>P. kochi</i> ( <i>P. knowlesi</i> and <i>P. inui</i> var. <i>cynomolgi</i> ).	Napier and Campbell (1932).
<i>S. irus</i>	<i>Plasmodium</i> sp. ( <i>P. knowlesi</i> and <i>P. inui</i> var. <i>cynomolgi</i> ).	Knowles and Das Gupta (1932).
<i>S. irus</i>	<i>P. knowlesi</i>	Sinton and Mulligan.
<i>S. irus</i>	<i>P. inui</i> var. <i>cynomolgi</i>	Sinton and Mulligan.
<i>S. nemestrinus</i>	<i>P. inui</i>	Leger and Bouilliez (1913); Bouilliez (1913).
<i>S. rhesus</i>	<i>P. inui</i>	Leger and Bouilliez (1912; 1913).
<i>S. rhesus</i>	<i>P. cynomolgi</i> ( <i>P. inui</i> var. <i>cynomolgi</i> )	Mayer (1908).
<i>S. rhesus</i>	<i>P. inui</i> var. <i>cynomolgi</i>	Sinton and Mulligan.
<i>S. rhesus</i>	? <i>P. kochi</i> ( <i>P. knowlesi</i> )	Napier and Campbell (1932).
<i>S. rhesus</i>	<i>Plasmodium</i> sp. ( <i>P. knowlesi</i> )	Knowles and Das Gupta (1932).
<i>S. rhesus</i>	<i>P. knowlesi</i>	Sinton and Mulligan.
<i>S. sinicus</i>	<i>P. inui</i>	Leger and Bouilliez (1912; 1913).
<i>S. sinicus</i> ( <i>M. radiatus</i> )	<i>Plasmodium</i> sp. ( <i>P. knowlesi</i> )	Knowles and Das Gupta (1932).
<i>S. sinicus</i>	<i>P. knowlesi</i>	Sinton and Mulligan.
<i>Silenus</i> sp.	<i>P. inui</i>	Halberstadter and Prowasek (1907).
<i>Silenus</i> sp.	<i>P. inui</i>	Mathis and Leger (1911).

### (3) Unsuccessful inoculation experiments.

#### Genus *Silenus* (*Macacus*).

<i>S. rhesus</i>	<i>Plasmodium</i> sp. ( <i>P. aemnopitheci</i> )	Chimisso (1922).
<i>Silenus</i> sp.	<i>P. pitheci</i>	Halberstadter and Prowasek (1907)

## APPENDIX II.

**THE NATURAL OCCURRENCE OF MIXED PLASMODIAL INFECTIONS  
IN *SILENUS IRUS* AND ITS SIGNIFICANCE.**

When we originally examined blood slides from specimens of *S. irus* found infected with malaria in nature, and in other specimens of the same species inoculated from them in this Laboratory, our first impression was that the plasmodial infection found was a mixed one. These infections were scanty, and it was difficult to determine definitely whether a mixed infection was present or not. On inoculation into local monkeys (*S. rhesus*), severe and usually fatal infections were obtained. In heavy parasitic infections of the latter animals no difficulty was experienced in determining the schizogony cycle of the *Plasmodium*; its duration proved to be a 24-hour one. The morphology of the parasite observed also appeared to be that of a pure infection in these animals, and to be identical with some of the forms seen in the natural host. On the whole, however, the morphological picture was very markedly different from that seen in *S. irus*.

When this apparent change in morphology was first noted, the most reasonable explanation appeared to be that the infection in the natural host was a mixed one. This was especially the case, as the forms seen in *S. rhesus* showed many of the peculiar features of those seen in *S. irus* (*vide* description of *P. knowlesi*).

Knowles and Das Gupta (1932) had previously reported such marked morphological variations, when a similar parasitic infection in '*Cercopithecus pygerythrus*' was transmitted to *S. rhesus*. These authors considered the possibility of a mixed infection as an explanation of their findings, but eventually concluded that the evidence available completely negated such an idea. Our results after passage of this infection through many specimens of *S. rhesus*, supported the occurrence of the morphological variations recorded by Knowles and Das Gupta (1932). In view of the evidence put forward by these workers against a mixed infection, their opinion that the parasite changed its morphology when inoculated into a host of another genus, seemed possibly correct. The work of Taliaferro (1932) with the monkey malaria of Panama, supported such a view.

We were prepared to accept the possibility of such a change in morphology in a single species of *Plasmodium*, more especially as we were unable at first to obtain any conclusive evidence of a mixed infection in the inoculated animals. When, however, the so-called '*Cercopithecus pygerythrus*' proved to be, not an African monkey, but an Oriental one of the genus *Silenus*, this support for the idea that the morphological variations were due to the different genera of the hosts disappeared.

After many trials we eventually succeeded in isolating, by blood inoculation from the same naturally infected host, *S. irus*, two morphologically distinct types

of *Plasmodium* in *S. rhesus*. On sub-passage these isolated strains remained true to type. The original host had a parasitic infection indistinguishable from that observed by us in the other specimens of *S. irus* ('*C. pygerythrus*'), which Colonel Knowles had kindly sent us. It also corresponded exactly with the descriptions given by Knowles and Das Gupta (1932) of infections in this species of monkey.

One of the species of *Plasmodium* isolated in *S. rhesus* was undoubtedly *P. inui* var. *cynomolgi* Mayer, 1907, while the other was definitely *P. knowlesi* sp. n., as described in this paper. These findings appeared to substantiate our original impression that the infection in the natural host was a mixed one. It was necessary, however, to obtain further confirmation of this, in view of the evidence which Knowles and Das Gupta (1932) had produced in support of the pleomorphic characters of their parasite.

A number of experiments were carried out with this object in view and the results are summarized below :—

(1) Passage of the isolated strain of *P. knowlesi* through normal specimens of *S. rhesus* produced no change in morphology.

(2) Similarly when a pure strain of this parasite was inoculated into a normal specimen of *S. irus*, the blood picture obtained was similar to that seen with this infection in *S. rhesus* and did not show the mixed character seen in the natural host.

(3) A similar absence of morphological variation was observed when *P. inui* var. *cynomolgi* was inoculated in the same way into normal specimens of *S. rhesus*.

(4) When a pure strain of *P. inui* var. *cynomolgi* was inoculated into a normal *S. irus*, the blood picture showed that parasite only and not the mixed morphology seen in the naturally infected hosts.

(5) When *P. knowlesi* was inoculated into a specimen of *S. rhesus*, which already showed a chronic infection with a pure *P. inui* var. *cynomolgi* strain, the blood picture obtained appeared to be identical with that seen in naturally infected *S. irus*, but *P. knowlesi* eventually predominated.

(6) When a pure strain of *P. inui* var. *cynomolgi* was injected into a specimen of *S. rhesus*, already showing a pure chronic infection with *P. knowlesi*, the parasitic picture obtained was at first a mixed one of the two Plasmodia, but later the former parasite markedly predominated. The superinfection proved to be comparatively mild and no treatment was necessary.

(7) When a normal specimen of *S. rhesus* was injected simultaneously with a pure strain of *P. knowlesi* and also one of *P. inui* var. *cynomolgi*, a similar blood picture was obtained in the early stages. *P. knowlesi*, however, quickly became predominant and death accompanied by hæmoglobinuria resulted.

(8) When one of the experimentally-produced mixed infections in *S. rhesus* was inoculated into another normal animal of the same species, it was possible to distinguish during the early stages the presence of both species of parasite,



but *P. knowlesi* very rapidly predominated and the monkey died with what appeared to be an acute infection with this parasite only.

Fuller details of these and other experiments will be given in a later paper.

In the light of these findings, the evidence produced by Knowles and Das Gupta (1932) against the possibility of a mixed infection, must be examined.

(a) The infections found in *S. irus* were all of the '*Cercopithecus*' type, while in *S. rhesus* they were all of the '*rhesus*' type. (In our opinion the '*cercopithecus*' type was a predominant infection with *P. inui* var. *cynomolgi*, while the '*rhesus*' type was one of *P. knowlesi*.)

(b) In *S. irus* ('*C. pygerythus*') all the infections were mild and the blood showed comparatively few parasites, while in *S. rhesus* the symptoms were very severe and the parasites very numerous (These findings correspond with the occurrence of a predominating infection of *P. knowlesi* in the latter species and of *P. inui* var. *cynomolgi* in the former.)

(c) They did not find that one or other species came to predominate at the expense of the other. (Our experience is that one usually predominates to the almost complete exclusion of the other, with the result that, unless one is very familiar with the differential characters of the two species, such mixed infections are very liable to be undetected.)

(d) They were unable to detect mixed infections by cultural methods. (Unless one is very familiar with the morphological features of the two species of *Plasmodium* for which a search is being made, it is very easy to overlook such mixed infections, more especially if one species is only present in scanty numbers.)

Our findings indicate that the parasitic pictures described by Knowles and Das Gupta (1932), and also seen by us in *S. irus* and *S. rhesus*, can be produced by mixed infections of *P. knowlesi* and *P. inui* var. *cynomolgi*. They also prove that in spite of several sub-passages neither of the pure strains isolated by us shows any detectable morphological variations when injected into simian hosts of different species.

It therefore seems to us that there is little doubt but that the apparent changes in morphology described in the parasites after injection into *S. rhesus*, were due to a mixed infection in the original host, *S. irus*. These apparent changes are almost certainly due to the usual predominance of *P. knowlesi* in *S. rhesus* and of *P. inui* var. *cynomolgi* in *S. irus*, in the types of monkey used by us.

The experience of several workers shows that malarial infections are not rare among monkeys in some localities in the tropics. Unless one assumes that there is only one species of monkey *Plasmodium* in each geographical area or a susceptibility in nature to only one species, the occurrence of mixed infections is to be expected, indeed its absence would be extraordinary, in the light of our knowledge of human malarial infections.

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The occurrence of such mixed infections in the lower monkeys does not seem to have been recognized previously. This condition may have a definite relationship to the very varied descriptions which have been given by different workers in recording Plasmodia in malarial infections in monkeys.

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\* We have attempted to include all important references to the malarial parasites of monkeys, even although not mentioned in the text.

† The date of this publication is probably 1913, although the date on the reprint and on the plate is 1912.

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EXPLANATION OF PLATE V.

*Plasmodium knowlesi* sp. n.

(From blood films stained by a 'panoptic' method.)

Fig. 1. Very early non-vacuolated parasites.

Figs. 2 to 7. Young ring forms (up to 3 hours growth); figs. 3 and 5 show accessory chromatin dot; figs. 4, 5 and 7 show chromatin in two masses of approximately equal size.

Fig. 8. Slightly older form (3 to 6 hours) showing slight irregularity of protoplasm.

Figs. 9 to 11. Larger ring forms (3 to 6 hours) with characteristic distortion of infested red cells.

„ 12 to 15. Older rings (6 to 9 hours) with more solid appearance and a few fine pigment granules.

„ 16 to 19. Solid, rounded trophozoites (9 to 12 hours); chromatin mass prominent and excentric; vacuole small or absent; pigment more abundant.

„ 20 to 23. Larger trophozoites with darker and coarser pigment (12 to 15 hours).

„ 24 to 26. Pre-segmenting forms showing commencing stippling of infested red cells and aggregation of pigment granules (15 to 18 hours).

„ 27 and 28. Early schizonts showing more definite stippling (18 to 21 hours).

„ 29 and 30. Advanced schizonts with aggregation of pigment into two large masses; infested red cells pale and irregular; more distinct stippling (21 to 24 hours).

Fig. 31. Mature schizont.

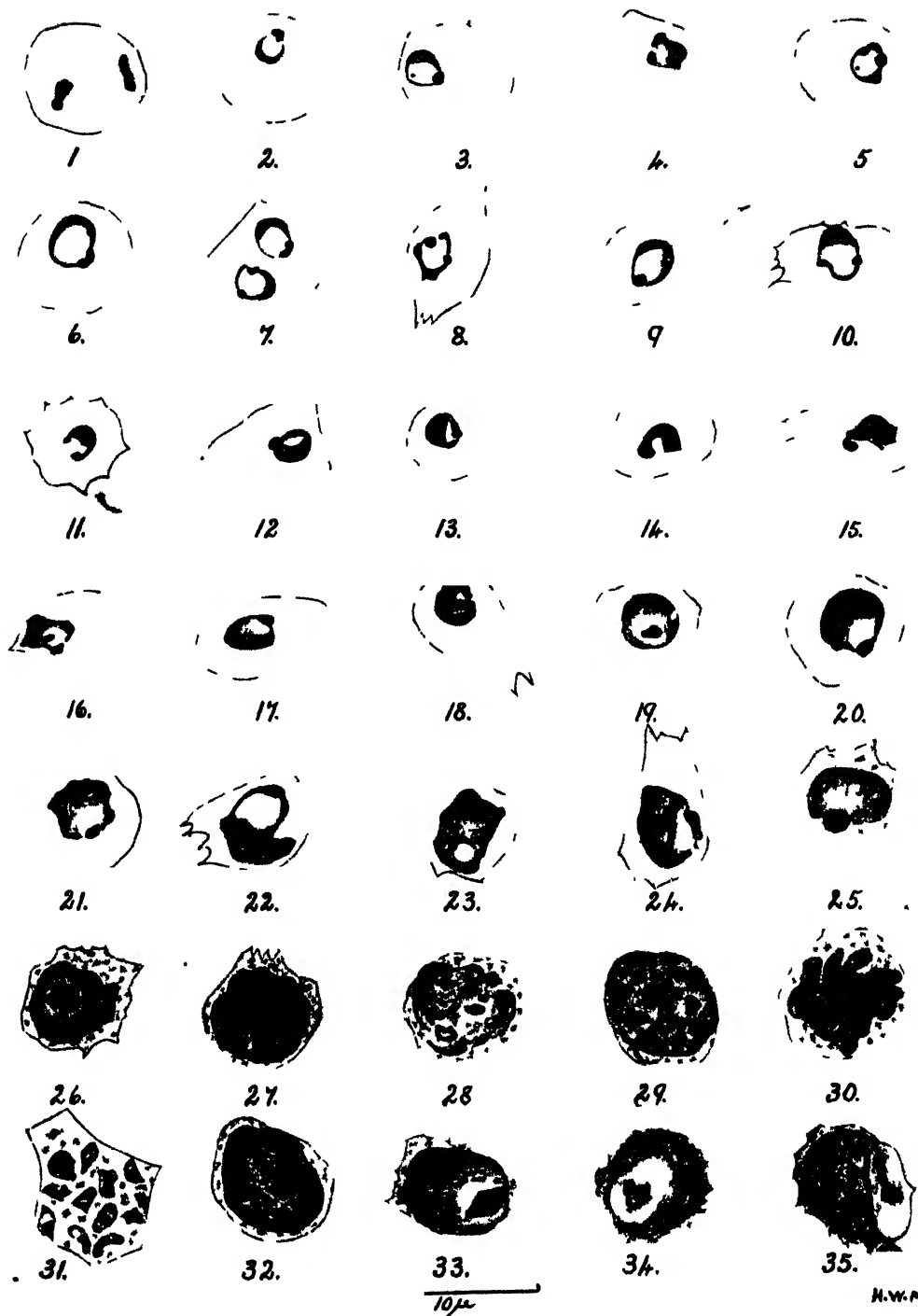
Figs. 32 and 33. Macrogametocytes.

„ 34 and 35. Microgametocytes.

(Note the characteristic deformities of many of the infested red cells.)

The times given in brackets indicate the approximate ages of the parasites.

# PLATE V







## PARENTERAL *VERSUS* ORAL ADMINISTRATION OF QUININE.\*

BY

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### I. INTRODUCTION.

As a rule the medical graduate in India, when he leaves the portals of his Alma Mater and enters the world of medical practice, does so with the impression that quinine by injection is much superior to oral administration, and that the intramuscular route is the most suitable method to use.

He has been taught that it does not entail the complicated technique of the intravenous route with which he, as a beginner, is not confident of success, and about the sudden fatal results of which, from paralysis of the cardiac mechanism (McCarrison and Cornwall, 1919; Brahmachari, 1922), he harbours exaggerated ideas. He has heard about the abscesses and tetanus arising from intramuscular quinine. His study of literature gives him the impression that these result from carelessness on the part of the medical practitioner, who accidentally injects the tetanus spores along with quinine (Manson-Bahr, 1929), or fails to sterilize adequately his syringe, his solution or the skin. He considers, however, that his technique is sufficiently good to prevent such obvious pitfalls. He has also read (Barker, 1911; Barnet, 1902; Dowler, 1902) and heard from his fellow practitioners about the unflinching success awaiting him if he administers quinine by this route. He, therefore, ignores the serious drawbacks of intramuscular quinine, and embarks upon this practice with preconceived ideas in favour of this mode of quinine administration.

When he actually starts his medical practice, he sees better results from injections as compared with unsupervised oral administration, and without

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\* This is part IV of a series of papers on malaria. The other parts have already been published (Karamchandani, 1928, 1929, 1932).

pausing to analyse the reasons, his ill-conceived ideas become fixed. The general preference on the part of the public for parenteral medication, and the resultant pecuniary benefit to himself, fog his vision still further. He continues this method of treatment until nerve paralysis, abscess, or tetanus disturbs his equanimity. He now refers to the literature on the subject, and finds a mass of this, including favourable reports by such authorities as Rogers (1918) and Stott (1915). Statements like 'no doubt that intramuscular method of giving quinine is far and away the best' (Kelly, 1907), 'injections are best given intramuscularly' (Anderson, 1910), 'injections of quinine hydrochloride give the best results' (Tobin, 1907), are again and again reiterated. Advice is given such as 'for subcutaneous and intramuscular use quinine acid hydrobromide, 7½–10 grains in 2 c.c., is preferable to other salts' (Editor, *I. M. G.*, 1926); 'quinine acid hydrobromide is stated to be the best salt for subcutaneous injection and being non-irritating is safe and suitable in practice' (Chinal, 1926); 'quinine bihydrobromide is the least irritating—I injected my thigh and the next day scarcely could find the place of puncture' (Moncrieff, 1907).\*

He tries another route, the subcutaneous one, as this does away with the danger of nerve paralysis and perhaps tetanus as well, leaving only the chance of occasional superficial necrosis. In India where malpractice is not adequately punished and the element of 'Kismet' is predominant, he considers that such a necrosis does not outweigh the apparent therapeutic and pecuniary advantages of this method. The vicious circle thus goes on uninterrupted. It is natural that the doctor—who perhaps is the only medical man in the place, as so often happens in rural places in India—when he meets with such an accident, will not broadcast the news, and thus the consensus of published opinion keeps the vicious circle going.

The author before undertaking these researches was a great advocate of the subcutaneous route for quinine administration (Karamchandani, 1929). For several years after his graduation in 1918 he practised intramuscular injections on a large scale without any apparent untoward results. However, in 1924 he was shown a case of paralysis of the musculospiral nerve after injection,\* under conditions which left him no doubt that the technique was aseptic. Almost simultaneously he saw a large slough in the arm of a lady who had had a quinine injection. The author therefore decided to abandon the use of intramuscular quinine. In attempting to find another route his attention was drawn to a statement by Martindale and Westcott (1928), that quinine hydrobromide was said to be entirely unirritating and well adapted for hypodermic injection. This technique was used for two years and seemed satisfactory. However in 1929 the author was shown two cases of skin necrosis produced by subcutaneous quinine administration. The practitioner responsible attributed the ill effects to the accidental intradermal injection of his solution and was not unduly perturbed.

\* A similar case was reported by Proctor (1926).

These results made the author critical of the method, but in view of his own results he decided that the lesions must be due to faulty technique. Absence from medical practice for some years prevented the continuation of this therapeutic method until the present experiments were started. Colonel Sinton pointed out to me in the words of Acton and Knowles (1924) 'that if those who advocate intramuscular quinine would but pause to try such injections experimentally in animals before they advocate the procedure in man', they would be much less certain of the harmless effects of such injections. A study of the effects of subcutaneous and intramuscular injections of quinine in rabbits was therefore undertaken with the results detailed here.

Before recording the result of these experiments, I wish to discuss all the aspects for and against quinine injections, because in my opinion this will clear the ground and make my case against quinine injections stronger, before I actually record the result of my experiments.

## II. EFFICACY OF PARENTERAL INJECTIONS OF QUININE AS COMPARED WITH ORAL ADMINISTRATION.

### (1) OPINIONS EXPRESSED BY OTHER WORKERS.

It is a general opinion (Phear, 1920), that 'quinine by the mouth is not one-tenth so efficacious as quinine injected' (Wilcox, 1919), and 'half a grain of quinine by hypodermic injection gives more certain results than 30-40 grains by the mouth' (Smith, 1911). Hudson (1922), as a result of his experiments in Sudan, considers quinine far more potent when given intramuscularly, and works on the assumption that 45 grains into the muscle is the equivalent of 80 grains by the mouth. In May 1921, the P. M. O., Federated Malay States, sent a circular to the officers in charge of all Government Hospitals, asking their opinion of the value of intramuscular injections. Their replies showed that nearly half of the officers were in favour of injections as a routine measure, except perhaps in the mildest cases; one doctor even went so far as to recommend their use in all cases of malaria whatever the type. Such opinions born of clinical experience of the two methods, though apparently a true indication, are not so in reality.

In the case of quinine injections, we prepare our solutions carefully, ensuring that they contain a proper quantity of quinine, and deliver the same into the system of the patient, while in the case of oral quinine we simply write a prescription and take it for granted that the medicine is taken by the patient. In this connection remarks by Sinton (1930) are worth recapitulating. He says 'in the case of quinine oral administration the usual procedure is to order a certain dose of the drug and then assume that this will be properly compounded, administered and retained by the patient, but unfortunately this is not so. The medicine however may not be of the strength prescribed, the patient may avoid taking it, or may not take the prescribed amount and, even if he has taken it, may not retain it. Several workers have discussed these fallacies

in detail, pointing that many of the stock medicinal solutions are not of the prescribed strength (Sinton, 1925; Megaw, Ghosh and Chatterji, 1928) and emphasising the necessity for ensuring that the drug is actually taken and retained by the patient (Fletcher, 1923; Sinton, 1926). In my opinion if as much care were taken to see that the patient received, retained and absorbed the prescribed amounts of the drug orally as is used for intramuscular injections as good or better results would be obtained'.

As Semple (1911a) remarks 'conclusions based on scientific facts are more likely to point in the direction of the truth than those based on opinions, probabilities or guess work'. It is, therefore, necessary to study this subject in the light of experimental work.

## (2) FATE OF QUININE IN THE BODY AFTER PARENTERAL INJECTION.

### (a) PRECIPITATION IN THE TISSUES.

#### (i) Previous work.

MacGilchrist (1911) studied the behaviour of quinine solutions when mixed with ox blood serum, and suggested the unlikelihood of quinine hypodermic injections being readily absorbed if given, as recommended by Squire (1908), in dilutions not more than 1 in 6, or the strength of Burroughs and Wellcome's quinine bihydrochloride 'vaporoles' which is one in five, or emergency quinine injections, which generally border on 1 in  $1\frac{1}{2}$ . MacGilchrist (1911) showed that a mixture of 0.1 gm. of quinine hydrochloride dissolved in 2 c.c. of boiling water (1 in 20), when cooled and added to two c.c. of ox blood serum, formed a deposit when allowed to stand. On analysis he found that slightly over half the quinine was in solution and slightly under half in the deposit—probably transformed (oxidized), combined with proteids and therapeutically useless. He says 'the importance of this observation in connection with hypodermic administration of quinine salts is obvious'.

His experience supports the work of Mariani (1904) on animals. Mariani found that, after injection of quinine bihydrochloride (1 in 5) into the leg muscle of a rabbit, 66.5 per cent of the amount of quinine injected could be recovered 17 hours later. Dudgeon (1919), however, could recover only traces of quinine from the muscles injected with concentrated solutions of quinine.

#### (ii) Fresh experiments.

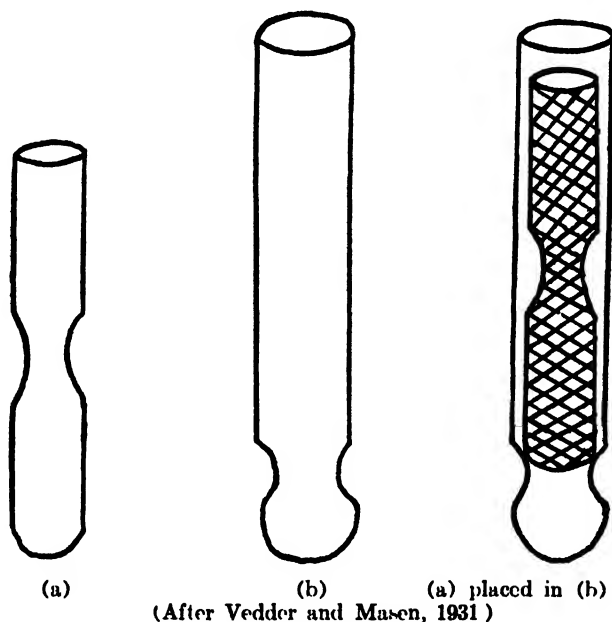
These are two conflicting findings, and to find out the truth for myself I repeated Mariani's experiments on rabbits.

In the experiments mentioned in this paper the method of Vedder and Masen (1931), for determination of quinine in the blood, was followed, with slight modification in the technique to make it applicable to extraction of quinine

from the muscle.\* The solutions and reagents were prepared according to the formulæ of Vedder and Masen (1931).

*Technique.*

0.5 gm. of quinine acid hydrobromide, in a one in three solution of distilled water, was injected into the gluteal muscles of a rabbit. After 17 hours the rabbit was killed and a portion of the injected muscle weighing 8.3 gms. was removed. This was minced well and then placed in a specially devised constricted tube (a), which had been previously packed with long fibre, acid-washed asbestos to within 3 c.c. of the top. The packing of asbestos, as suggested by Vedder and Masen, was neither too tight, thereby interfering with



ether percolating through, nor too loose thus allowing escape of fine muscle fragments to the bottom of the tube into the ether. This tube was then inserted into another tube (b), and 5 c.c. of ether was poured over the top of the tube (a) and allowed to percolate into the outer tube (b). The extractor was

\* It may be remarked that previous workers suffered from a handicap, because they extracted the alkaloid with ether after adding concentrated ammonia, with a view to rendering quinine soluble in the ether. Ammonia causes troublesome emulsions from which ether separates slowly or not at all, so that it is impossible to recover added quinine quantitatively (Vedder and Masen, 1931). Although this method was improved by Ramsden and Lipkin (1918), who used hot ammonium sulphate solution for extraction of the alkaloid, from which quinine was later extracted with ether after alkalization with concentrated ammonia, still Acton and King (1921) found that losses in recovery varied from 15-35 per cent, and they therefore made an allowance of a constant loss of 25 per cent.

then connected with a reflux condenser and immersed in a warm water bath up to the level of ether in tube (b). Extraction was allowed to proceed for 4 hours. The inner tube (a) was then removed, and the outer tube (b) containing the ether extract placed in boiling brine bath and evaporated to dryness. Five c.c. of 2 N sulphuric acid saturated with zinc sulphate was added and the tube returned to brine bath for three minutes to facilitate solution of quinine. This solution was next filtered while hot through a No. 42 Whatman filter paper and the filtrate allowed to cool to room temperature.

An aliquot portion of the filtrate, 3 c.c., was measured and put in a tube marked (x), and three standards prepared as follows:—0.01 gm. of quinine acid hydrobromide was weighed and dissolved in 50 c.c. of 2 N sulphuric acid saturated with zinc sulphate. This formed the stock standard, from which 1, 2, and 3 c.c. were measured, poured in tubes marked w1, w2, w3, and diluted with 9, 8, and 7 c.c. respectively of 2 N sulphuric acid saturated with zinc sulphate. These formed the working standards, 5 c.c., of which contained 0.1, 0.2, 0.3 mgm. quinine respectively. Three c.c. of each of the standard quinine solutions from the tubes marked w1, w2, w3 were measured and put in three tubes marked t1, t2, t3. To each of the four test tubes, *viz.*, t1, t2, t3 and (x), were added 0.06 c.c. of gum arabic solution, followed by same amounts of the potassium bismuthous iodide reagent. After mixing, comparison was immediately made in a colorimeter with the standard set at 10 mm., and completed within 2 minutes from the time that solutions were mixed. The calculation was made according to the following usual colorimetric formula:—

$$\text{Concentration of the unknown} = \frac{\text{Reading of the standard} \times \text{concentration of the standard.}}{\text{Reading of the unknown.}}$$

### *Results.*

Out of 7½ grains of quinine injected 6 grains were recovered, *i.e.*, 80 per cent. The control yielded almost cent per cent quinine. The claims of Vedder and Masen, that this method contains an average error of 3.5 per cent and which also is not due entirely to incomplete extraction, thus stand fully substantiated. Mariani's experiments are confirmed by this work.

#### (b) ABSORPTION OF QUININE AFTER INJECTION.

It is however argued that quinine combined with proteids and thrown into a coagulum is not lost, but is gradually liberated from the coagulum for 2 or 3 days after injection (Rossabach, quoted by MacGilchrist, 1911). The protagonists of this theory have turned this point to their advantage, and Smythe (1906) thinks that 'after hypodermic injections, quinine is slowly absorbed and eliminated for weeks, that 20 grains injected in the flanks give protection from malaria for a month at least'. Kleine (1899) also came to the conclusion that 'quinine was precipitated largely at the site of injection, and that quinine was

slowly absorbed from this depot, which lasted for weeks—a useful method for prophylaxis of malaria'.

This slowness of absorption after quinine injection, as compared with oral quinine, is also stressed by Megaw (1907), who has shown that 'the temperature of malarial patients takes about 12 hours longer in coming to normal when treated with hypodermic method than when quinine is given by mouth'. Scott (1907) also subscribes to this view and assumes that 'the absorption is slower from the subcutaneous tissues than from the mucous membrane of the gastric tract'.

**(i) Elimination of quinine in the urine after oral administration as compared with parenteral injections.**

A smaller proportion and a tardier elimination of quinine in the urine, when administered subcutaneously as compared to that by mouth, is another indication of slow absorption by the former route. The following give the proportion eliminated in the first 24 hours in urine after administration (1) by mouth and (2) subcutaneously :—

Kleine (1901)	..	..	by mouth (fasting)	21·91
			subcutaneously	12·13
Mariani (1904)			Do.	24·7
			Do.	14·74
			Do.	19·50
Schmitz (1907)	..	..		=
			Do.	17·9
			Do.	25·8
Giemsa and Schauman (1907)			Do.	21·6

Although there is little harmony in the above results regarding the proportion of quinine eliminated in urine after hypodermic injection, still all are in agreement that the proportion of quinine eliminated when administered subcutaneously is much less than when given by mouth.

Mariani (1904) after injecting 1 gm. of quinine bihydrochloride dissolved in 10 c.c., and the same amount in 2 c.c. of water, also found that maximum elimination of quinine in the urine occurred between 6–12 hours and 9–18 hours respectively. After oral administration, MacGilchrist (1917a) found that after fasting, the maximum elimination of quinine occurred between 3–7 hours, and during or soon after meals between 6–12 hours.

It must be remarked here that the amount of quinine absorbed must be equal to the amount of quinine eliminated in the urine plus the amount that has undergone cleavage in the body. The determination of the amount of quinine eliminated in urine cannot possibly give us any idea of the amount absorbed, unless we are able to determine at the same time the amount that has undergone cleavage. Further there being no reason to suppose that the quinine which undergoes cleavage is of no therapeutic value before cleavage has occurred.

The inference that greater elimination in the urine connotes greater therapeutic value is unwarranted. But, since there is no means of estimating the amount of quinine that has undergone cleavage in the body, tardier and lesser elimination in the urine after hypodermic injections of quinine, supported by actual clinical results of these two methods (Megaw, 1907; Scott, 1907), would appear to be in favour of the oral route.

Again, because after intravenous injection the elimination of quinine in the urine is tardier and less in amount, as seen in the averages tabulated below from Mariani (1904), is one justified in concluding that the intravenous route is less efficacious than the oral one ?

		Intravenous injection. Per cent.	Intramuscular injection. Per cent	Oral (fasting) administration. Per cent.
1st day	..	20.54	18.47	24.70
2nd day	..	6.33	9.6	?
3rd day	..	1.07	4.9	?
4th day	..	..	2.45	?
TOTAL	..	27.94	35.43	40.80

### (ii) Concentration of quinine in the blood.

The conclusions of Maxcy (1928) may fittingly be quoted here :—

'Morgenroth (1918) estimated that quinine content of the blood was 1 in 20,000 a few minutes after intravenous injection, and 1 in 150,000 after oral administration of therapeutic doses. Disappearance of the drug from the blood is very rapid; Hatcher and Gold (1926) could not detect its presence in individual specimens 30 minutes after oral or intramuscular administration. It is not merely excreted, for 25 per cent can thus be accounted for, the rest being broken up into simpler substances, a process for which the liver seems to be largely responsible. Kirschbaum (1923) has produced malarial infection by injecting blood which has been set aside for 24 hours after being mixed with quinine to the extent of 1 in 10,000, a concentration far greater than has ever been maintained in the circulating blood. Again in certain cases which they investigated, Ramsden, Lipkin and Whitley (1918) found the quinine content to be highest, and cinchonism greatest in those in whom parasites persisted longest. Clearly then, quinine as such is not plasmodicidal. But of the effect of quinine in curing malaria there is no question at all, and we must suppose that it exercises its striking action in some indirect way. We have to recognize that intravenous quinine does not act by suddenly making available a large amount of parasitocidal substance. And if the liver is the organ where quinine is not only rapidly snatched from the circulation but most effectively turned into the plasmodicidal substance—whatever that may be—then quinine medication does not seem to lead to the desired result as directly as administration *via* the mouth and portal vein. Intravenous injection of quinine does not possess any superiority *per se* over ordinary administration by mouth and the method is not without danger to the patient'.



Acton took 1 gm. of quinine base (as bisulphate in an aqueous solution) by mouth, had his blood examined at hourly intervals and tested for quinine content. These estimations showed the following concentrations (Acton and King, 1921) :—

Interval, in hours, after administration.	Concentration of quinine in blood.	Amount in blood mg.	Percentage of dose taken.
1	1 in 150,000	33	3·3
2	1 in 187,000	27	2·7
3	1 in 225,000	22	2·2

The concentrations reached in blood after oral administration of smaller doses of quinine were the following :—

Dose.	Concentration after one hour.	Concentration after two hours.
0·5 gm.	1 in 375,000	1 in 500,000
0·25 gm.	below 1 in 375,000	below 1 in 750,000

#### *Relationship of concentration to therapeutic effects.*

Acton (1921) has pointed out 'that small doses of quinine (1–3 grains) are insufficient to control fever because the concentration attained in the blood, 1 in 500,000, i.e., 2 mg. per litre or less, is probably non-toxic to the parasites. Also a single large dose (30–90 grains) is not sufficient to effect a permanent cure because the concentration of alkaloid circulating in blood must be under "the certain lethal limit". A cure in malarial fevers only occurs after the treatment has been continued for some time (3–6 weeks), i.e., if quinine concentration in the blood has been maintained between the limits of 1 in 150,000 and 1 in 250,000'. We know that when the patient is placed on a course of 30 grains of quinine *per diem*, the parasites rapidly disappear from the peripheral blood. Since 'the rate of destruction for each cycle of benign tertian parasite has been calculated to be over 90 per cent of the parasitic population' (Acton, 1921), the probability is that quinine is acting on the malarial parasite in sub-lethal concentrations. 'We know that the malarial parasites differ from each other as regards their site of multiplication. The malignant tertian parasites multiply in the deeper vessels, i.e., mesentric; and in heavy infections sporulation may occur in other sites, e.g., brain capillaries. Schizonts are rarely seen in peripheral blood. The benign tertian parasites multiply anywhere in the blood stream, and schizonts are usually found in the peripheral blood before the attack of fever, whilst in quartan infections schizonts are numerous and

invariably found in the peripheral blood. The sterilizing value of quinine in these three infections varies in the following order of efficiency:—90 per cent or over of cures in malignant infections, 25 per cent in benign infections, and 15–20 per cent in quartan infections. The higher concentration of quinine in the portal vessels acting on the site where merozoites are set free, may possibly be the factor concerned with the enhanced rate of cures in malignant tertian infections' (Acton, 1921). If apart from concentration, accessibility be the main physical condition concerned in the destruction of the malarial parasite by quinine then administration by mouth and *via* the portal vein should possess superiority *per se* over intravenous route in malignant tertian infections at least, specially so when 90 per cent of quinine disappears from the peripheral blood within 5 minutes of the injection by the vein.

Vedder and Masen (1931), while discussing the results of their experiments with 'the determination of quinine in the blood as guide to the treatment of malaria', point out that

'in a number of patients a very considerable concentration of quinine in the blood was reached within half an hour after administration and in most cases within one hour after the administration of quinine. This demonstrates the ease with which even an insoluble salt (sulphate) of quinine is absorbed after administration by mouth, when given after a meal. Intravenous injection can at most save only a short time in the attainment of the maximum concentration in the blood, and several observers have found that the majority of the injected quinine disappears from the blood within 15–20 minutes. Administration by mouth is, therefore, unquestionably the preferred method; there is apparently no excuse for intramuscular injections, and intravenous injections should be used only in very small number of cases in coma that cannot take quinine by mouth. Even in these cases it is probable that quinine should be introduced into the stomach with a tube in order to maintain the concentration of the blood, because a single dose of 10 grains never produces the maximum obtainable in the blood, and practically in every case, higher concentrations were obtained in the afternoon after the second dose of 10 grains'.

The final conclusion then is that oral quinine is not in any way inferior to parenteral quinine, and 'oral quinine, of the various methods of quinine administration, is the most practical for routine use, and gives as good results as the others' (Rennie, Acton, Curjel and Dewey, 1920).

### (iii) Prolonged therapeutic effects of parenteral quinine.

Regarding the belief that parenteral quinine provides a reservoir, whence the drug is slowly and continuously absorbed thereby maintaining a steady concentration of quinine in the blood. Kleine (1902) himself, who first propounded the theory of quinine reservoir, on finding that elimination in urine was so small in amount and that elimination did not continue so long as he had formerly supposed, concluded that subcutaneous administration of quinine was of less therapeutic value than the oral one.

Fletcher (1923), in a series of cases, did not find that quinine was excreted over a longer period when injected than it was when administered by the mouth. By whichever route it was given, quinine usually disappeared from the urine

in about 26 hours, *i.e.*, it was no longer detected in the unconcentrated urine by addition of Mayer's reagent. His remarks may well be reproduced here. He says

'we are unable to discover the grounds for this belief (*i.e.*, quinine forms a reservoir when given intramuscularly) but its origin may be a note published by Colonel John Smythe in 1906. He recommended that 20 grains of quinine should be injected into the flanks and stated that, if this method were employed, the individual may count on having, for the next month at least, enough quinine in his blood to keep him safe; without the annoyance of the head symptoms which so commonly result from a large dose given by mouth. He based this recommendation on the case of a child, in whose urine he found an appreciable quantity of quinine 21 days after she had received two injections (10 grains each) of quinine bisulphate. This was a remarkable occurrence; but like the incident of Elisha and the widow's cruse, it did not take place under strict experimental conditions'.

### (3) POINTS TO BE OBSERVED IN TESTING THE COMPARATIVE THERAPEUTIC EFFICIENCY OF ORALLY ADMINISTERED QUININE.

The evidence so far adduced, then, points to the fact that absorption is no better after hypodermic than after oral administration, and, to obtain the optimum action of quinine when administered orally, it is important that the drug should be administered, retained and absorbed properly.

#### (a) ADMINISTRATION OF THE PRESCRIBED DOSAGE.

Those of us who have had stock quinine solutions tested, know only too well how often the solutions of quinine are found to be weaker than the prescribed strength. At present, when quinine is literally worth its weight in silver the temptation for adulteration by the subordinate staff is great. 'Even pills and tablets may fail to contain the amount of the drug stated to be present' (Sinton, 1930). The simple methods of testing quinine solutions devised by Sinton (1925), and Megaw, Ghosh and Chatterji (1928) should therefore be made full use of.

A second point about administration is the use of citric acid as a solvent of quinine. It has been pointed out by Sinton and Baily (1924) that there is a varying degree of acidosis associated with malarial attacks. If, therefore, a mineral acid is prescribed, this condition will be increased. Such an eventuality is avoided by the use of citric acid because this acid is absorbed in the system as an alkali. Gastric irritation and renal irritation, following prolonged use of mineral acids, is another drawback from which an organic acid like citric acid is free. Finally, owing to the bitter taste of quinine, supervision of administration by a reliable man is very important. If the above facts were realized by the medical officers, their reticence regarding personal supervision of quinine parades, the abrogating and consigning of these to the subordinate staff would disappear.

#### (b) RETENTION OF THE PRESCRIBED DOSE

Even after the drug is taken it is not certain that it will be retained. The army practice (Stott, 1916) of ordering patients to call out their names after

taking quinine, is not without fallacy, for this may be a proof of swallowing but not ensuring that it will not be vomited. Sinton's practice of giving quinine after food to prisoners and sepoys will do away with voluntary vomiting, because these people are loath to induce vomiting and thus lose their meal as well. Regarding involuntary vomiting, Sinton is of the opinion that this is probably due to 'acidosis' and that it is an attempt on the part of the system to restore pH balance by expelling the acid contents of the stomach. Sinton's alkaline mixture is ideal in such cases, for it not only aids the therapeutic effect of quinine, but also serves as an alkaline lavage of the stomach, and thereby ultimately checks the symptoms. 'In severe cases of vomiting 20 minims of adrenalin solution (1 in 1,000) by mouth has never failed to check this symptom' (Sinton, 1930).

#### (c) ADJUVANTS TO ABSORPTION OF QUININE.

Quinine may be taken and retained, but it may not exercise its optimum therapeutic effect, unless it is properly absorbed. The alimentary mucosa may be coated with mucus, it may be disordered on account of diarrhoea or constipation, or again it may be congested and irritable as the result of malarial attack. All these factors hinder absorption of quinine, hence the value of preliminary administration of calomel and saline, and subsequent purgation. This is one of the reasons why Sinton's quinine mixture contains drachm one (4 gms.) of magnesium sulphate per dose. MacGillchrist (1911) attributes the greater efficacy of Warburg's tincture, to its purgative and aromatic contents, *viz.*, aloes, rhubarb, and gentian. The flow of bile and the effect of purgation help to relieve acidosis, and are other advantages in favour of the procedure in malaria (Sinton, 1923).

Mention has been made above regarding acidosis in malaria and the advantages of Sinton's alkali mixture. The ancient practice of giving alkalies to enhance the action of quinine (James, 1922) has now been put on a scientific basis by the researches of Sinton. It is an important adjuvant, which when universally used with quinine treatment, will end the routine practice of quinine injections. Acton and Chopra (1926) confirmed this enhanced action of quinine. They showed that, by increasing the degree of alkalinity in the intestines, there was a greater diffusion of quinine into the circulating blood, and so the concentration attained in the blood was higher when alkalies were administered before or with quinine. Again these authors state 'we may say that the quinine molecule is more diffusible in an alkaline than in an acid substrate. It attains a concentration which is probably sublethal to the parasites. In sublethal concentrations quinine hinders the movement of these parasites, so that they fail to reach their food supply. Of more mature forms of the trophozoites it probably hinders reproduction by the formation of smaller number of merozoites. The young parasites that are adherent in semi-torpid state on the red blood cells are swept away by the friction of the blood stream. They lose their food supply, which they get from the red blood cells and die

of starvation in the tissues of the spleen, etc. The parasites are digested by cytolytins which are derived most probably from the reticular endothelial tissue'.

#### (4) CONCLUSIONS AS TO THE COMPARATIVE THERAPEUTIC VALUES OF ORAL AND PARENTERAL ADMINISTRATION OF QUININE.

It is clear then that the view, that quinine should be injected because it has greater therapeutic value than when given by the mouth, is incorrect. This question has been discussed several times before and, although the conclusions reached have been in favour of the oral route, it has made little difference in actual practice. Manson (1914) taught that 'intramuscular injection must not be practised without good reason, or as the routine treatment of ordinary malarial attacks'. Ross (1914, 1914a, 1921) on many occasions has expressed himself strongly against the use of injections in ordinary cases of malaria; he considers that they are necessary only in grave seizures. James (1918) states 'when quinine can be given by mouth, and can be absorbed by that route its action is as effective as when it is given intravenously or intramuscularly and it should be given by those routes only when it cannot be given by mouth'. Bass (1921) objects to intramuscular quinine because it is inferior therapeutically. Dixon (1920) stated that he supposed every one agreed that the intramuscular injection of quinine was inferior to quinine by the mouth.

### III. RELATIONSHIP BETWEEN THE STRENGTH OF QUININE SOLUTION INJECTED AND THE REACTION OF THE TISSUES.

#### (a) REPORTS OF PREVIOUS WORKERS

The next point to be considered is the relationship of the strength of the quinine solutions employed for intramuscular injections to the degree of (1) necrosis and (2) suppuration of the tissues into which they are injected. Although this fact has been emphasised so often, yet the majority of medical men do not realize it, otherwise statements would not be made like the following: 'It is possible to inject an effective dose of quinine into the muscle *thousands of times* without any induration or pain on movement, much less any sloughing' (Bennet, 1915); or 'even formation of painful nodes I now regard as a failure in technique' (Stott, 1915). Dudgeon (1919) writes 'I have discussed the question of quinine necrosis with innumerable medical officers who have had wide experience of intramuscular injections of quinine, and it is by no means uncommon to learn from them, that they were unaware, that such effects occurred in the tissues apart from negligence'. That necrosis does occur there is no doubt. As Acton (1921) has said 'burying the needle deep into the muscles and fondly imagining that no injury to the tissues occurs, is similar to the illusion that affects the ostrich when it buries its head in the

sand'. The author once subscribed to the view that quinine acid hydrobromide when injected aseptically caused no necrosis of the tissues, and when he started experiments on rabbits it was no little surprise to find the lesions recorded below.

(b) FRESH EXPERIMENTAL EVIDENCE.

(i) **Salts of the cinchona alkaloids used.**

The following salts of the cinchona alkaloids were selected for experiments:—

(1) *Quinine acid-hydrochloride*.—This salt is almost universally used on account of its easy solubility, 1 in 1 part of water, and because it contains 74·8 per cent of anhydrous quinine base.

(2) *Quinine acid-hydrobromide*.—This salt is reported to be the least irritant and therefore preferable, not only for intramuscular but also for subcutaneous injection (Editor, *I. M. G.*, 1926; Martindale and Westcott, 1928). It is soluble 1 in 7 of water and contains 60 per cent of anhydrous quinine base.

(3) *Quinine alkaloid*.—This was dissolved in dilute hydrobromic acid with pH adjusted to that of quinine acid-hydrobromide solution, and used at the suggestion of Col. Sinton.

(4) *Quinine urea-hydrochloride*.—This salt is also advocated in 10 grain doses for adults, subcutaneously (Editor, *I. M. G.*, 1926). It is soluble 1 in 1 part of water, and contains 70 per cent of the base.

(5) *Cinchonine acid-hydrochloride*.—This salt has been recommended by Rogers (1918) as not causing any necrosis of the tissues.

(6) *Cinchonine acid-hydrobromide* has similarly been recommended by Rogers (1918).

(ii) **Results of experiments.**

RABBIT NO. (1).—Wt. 2,590 gms. Quinine acid-hydrobromide 0·1 gm. in 0·75 c.c. of water injected subcutaneously. Killed after 21½ hours.

*Result*:—Congestion at the site of injection, no necrosis.

RABBIT NO. (2).—Wt. 2,000 gms. Quinine acid-hydrochloride 0·1 gm. in 0·75 c.c. of water injected subcutaneously. Killed after 72 hours.

*Result*:—Marked œdema with necrosis and hæmorrhages 2 inches in diameter. Control injected with sterilized water showed nothing.

RABBIT NO. (3).—Wt. 1,880 gms. Quinine acid-hydrochloride 0·1 gm. in 1·5 c.c. of water injected subcutaneously. Killed after 72 hours.

*Result*:—Marked œdema with necrosis of the subcutaneous tissues and commencing necrosis of the skin. Control showed nothing.

RABBIT NO. (4).—Wt. 2,040 gms. Quinine acid-hydrobromide 0·1 gm. in 1·5 c.c. of water injected subcutaneously. Killed after 72 hours.

*Result*:—Intense œdema with sloughing of the subcutaneous tissues extending over an area of 2 inches square. Surrounding congestion

with ecchymosis. pH of this quinine solution was 3.454. Control injected with sterilized water showed nothing.

**RABBIT NO. (6).**—Wt. 1,870 gms. Quinine alkaloid 0.1 gm. in 1.25 c.c. of dilute hydrobromic acid (pH 3.145) injected subcutaneously. Killed after 72 hours. *Result* :—Edema with necrosis of the subcutaneous tissues, and commencing necrosis of the skin. Control of 75 per cent dilute hydrobromic acid showed nothing.

**RABBIT NO. (7).**—Wt. 1,820 gms. Quinine acid-hydrobromide 0.1 gm. in 1.5 c.c. of water injected subcutaneously. Killed after 72 hours. *Result* :—Except for very slight congestion with ecchymosis, nothing was found in the subcutaneous area. On cutting into the muscle a patch of necrotic muscle tissue was found, 1 inch wide and  $\frac{3}{8}$  inch deep. *Note*—In this case the injection seemed to have been made into the muscle inadvertently.

**RABBIT NO. (8).**—Wt. 2,100 gms. Quinine acid-hydrobromide 0.1 gm. in 3 c.c. of water injected subcutaneously. Killed after 72 hours. *Result* :—Edema of the subcutaneous tissues about 1 inch in diameter. No necrosis. Cutting into the muscle revealed an area of slight necrosis involving only the superficial layer. Control not done in rabbits Nos. (7) and (8) because it was considered unnecessary.

**RABBIT NO. (9).**—Wt. 1,970 gms. Quinine urea-hydrochloride 0.1 gm. in 0.75 c.c. of water injected subcutaneously. Killed after 72 hours. *Result* :—Area of necrosis of the muscle (into which quinine seemed to have been injected)  $1\frac{1}{2}$  inches  $\times$   $\frac{1}{2}$  inch with congestion extending over an area  $2\frac{1}{2}$  inches long and 1 inch wide at one end and  $\frac{1}{2}$  inch at the other. The area affected presented the appearance of coagulative necrosis.

**RABBIT NO. (10).**—Wt. 1,950 gms. Quinine urea-hydrochloride 0.1 gm. in 1.5 c.c. of water injected subcutaneously. Killed after 72 hours. *Result* :—Diffuse edema extending over an area  $2\frac{1}{2}$  inches in diameter, with slight necrosis of the subcutaneous tissues about  $\frac{1}{4}$  inch in diameter in the centre. Cutting into the muscle revealed nothing.

**RABBIT NO. (11).**—Wt. 2,110 gms. Cinchonine acid-hydrochloride 0.1 gm. in 0.75 c.c. of water injected subcutaneously. Killed after 72 hours. *Result* :—Nothing in the subcutaneous area but on cutting into the muscle at the site of injection an area of sloughing and suppuration 1 inch  $\times$   $\frac{3}{4}$  inch in diameter was discovered within the muscle tissue. The injection seemed to have been made into the muscle.

**RABBIT NO. (12).**—Wt. 1,760 gms. Cinchonine acid-hydrochloride 0.1 gm. in 1.5 c.c. of water injected subcutaneously. Killed after 72 hours. *Result* :—Wide-spread area of congestion and edema of

the subcutaneous tissues 3 inches square, with an area of commencing necrosis of skin  $\frac{1}{2}$  inch in diameter.

RABBIT NO. (13).—Wt. 2,150 gms. Cinchonine acid-hydrobromide 0.1 gm. in 0.75 c.c. of water injected subcutaneously. Killed after 72 hours. *Result* :—Congestion of the subcutaneous tissues 2 inches  $\times$  1 inch with necrosis of only the superficial layer of muscle 1 inch  $\times$   $\frac{1}{2}$  inch.

RABBIT NO. (14).—Wt. 1,950 gms. Cinchonine acid-hydrobromide 0.1 gm. in 1.5 c.c. of water injected subcutaneously. Killed after 72 hours. *Result* :—Edema of the subcutaneous tissues 2 inches in diameter, with congestion extending over an area 3 inches in diameter. Slight sloughing was also present in the centre.

Doses of 0.1 gm. dissolved in 0.75 c.c. and 1.5 c.c. of water respectively were used with a view to finding out if there was any difference between the escharotic effect produced by these two dilutions. MacGilchrist (1911) thinks that the more dilute the solution of quinine, the greater its absorbability, and he therefore recommends weak solutions. Brodrib (1922) reports that since he adopted the injection in dilute solutions of quinine (grains 5 in 10 c.c.) he saw no induration follow the injection, and that it was safer and more effective. Dudgeon (1920) however thinks that the more dilute the quinine solution the greater the trauma and therefore the danger of sloughing. He says 'the results showed that no advantage was gained by injecting the quinine in twice the quantity of saline'.

It will be seen from the above experiments that higher dilutions of quinine acid-hydrobromide solutions [1.5 grains in 3 c.c. of water, rabbit No. (8)] produced less necrosis when injected subcutaneously. The author is inclined therefore to agree with MacGilchrist (1911) and Brodrib (1922) and recommend this dilution in those very rare instances when such injections are necessary (*vide infra*).

The author also wishes to draw attention to rabbits Nos. (1), (4) and (7) all of which were given injections of quinine acid-hydrobromide. No. (1) showed no necrosis at all, but this cannot be compared with the other two as it was killed after 21 $\frac{1}{2}$  hours. In the case of rabbits Nos. (4) and (7) all the factors were almost identical, still in the case of rabbit No. (4) there was an intense reaction with considerable sloughing, while in the case of rabbit No. (7) the reaction was not so intense. This shows that even after careful sterilization and injection of quinine solutions under identical conditions, there are liable to be differences in the reaction, due to causes over which we have no control. The only possible explanation the author can give for this apparent incongruity, is that perhaps the needle of the syringe in the case of rabbit No. (4) punctured some vein which produced hæmorrhage in the subcutaneous tissues. Finally it will have been noticed that injection of 1.25 c.c. of 75 per cent dilute hydrobromic acid in rabbit No. (6) showed no reaction at all.



## (c) DISCUSSION OF EFFECTS.

It may be argued that quinine experiments with rabbits are not a true index of what happens in man. Stott (1915) was perhaps the first to suggest this when he criticized the well-known experiments of Semple (1911). His argument was that quinine experiments with small animals should be received with extreme caution, because in the first place the majority of these animals are herbivorous in nature, hence their blood being alkaline, the reaction after injection of acid solution would be greater than in carnivorous man. Secondly as a rule the dose of quinine used per gramme body weight of these small animals would seem to be enormous. A special article in the *Indian Medical Gazette* (Criticism, 1911) also criticizes Semple's memoir and his results are thought to be fallacious because (a) rabbits are not carnivorous animals and (b) the dose given to these animals would be equivalent in man to 210 grains quinine dissolved in 4 ounces of water.

Two points are raised in this controversy (1) rabbits are not carnivorous like man, therefore their blood, being more alkaline than that of man, will show greater reaction on the injection of an irritating acid solution, and (2) the dose of quinine used to bring about the reaction in rabbits by weight is proportionately far in excess of that in man. Dealing with the first point, the reaction of human blood is not more acid—the difference being insignificant—and if his pH goes below 7 the man dies. Besides man is not necessarily a carnivorous animal. The Brahmans of the Hindu community have not taken flesh for generations and still the pH of their tissues is the same as that of other communities. Quinine experiments are recorded (Dudgeon, 1919) in frogs with doses suitable for their body weight (0.0004 gm. quinine acid-hydrochloride in 0.1 c.c. of saline for a frog of 60 gms. weight) and the results have been 'extensive muscular necrosis, abundant hæmorrhages and widespread inflammation'. Finally it may also be mentioned that injection of a control acid solution in rabbit No. (6) showed no reaction at all.

Regarding the second point, my doses in the rabbits would be equivalent to 50 grains for a man and since 10–15 grains is the ordinary dose for a man, the doses given to rabbits would work about four to five times greater. If the extent of the lesions in my rabbits were reduced to  $\frac{1}{5}$ th, there still would be well marked area of necrosis and hæmorrhages.

Those to whom this argument does not appeal, I would refer to the experiments by Dudgeon (1919). He gave quinine injections in doses of 1.25 gms. of the acid-hydrochloride salt in 2.5 c.c. of saline to large animals like horses and mules, and found 'very extensive gelatinous œdema with localized necrosis'. The weight of these non-carnivorous animals, as compared to that of man, is at least 20 times greater and the dose therefore as compared to man was much less (about  $\frac{1}{5}$ th), still well marked lesions occurred. But the most convincing testimony of necrosis occurring from quinine injections (if recorded cases of abscesses and sloughing from quinine injections are omitted as being due to

imperfect sterilization) comes from the record of various workers who have examined the human muscle injected with quinine :—MacGilchrist (1917b), one case; Dudgeon (1920), 8 cases; Figdor and Pinnock (1922), one case, and Fletcher (1923), 15 cases. Before leaving the subject of necrosis, the conclusions of Acton and Chopra (1924) may be mentioned that 'injections of the alkaloid salts of cinchona into the muscle of man should be considered malpraxis, as this tissue is unable to survive the presence of these bases at a high concentration, *viz.*, 1 in 2,000'.

Necrosis due to direct protoplasmic poisoning effect, which may involve important nerves and blood vessels, is not the only result of quinine injections. It may initiate extensive suppuration. Formerly this suppuration was considered to be due to introduction of organisms at the time of injection, but now we know that it can be the result of infection with bacteria carried to the injured tissues by the blood. Rous and Jones (1916) have conclusively shown that living phagocytes are able to protect certain ingested organisms from the action of destructive substances in the surrounding fluid, and even from a strong homologous antiserum. If now an infective agent can be 'walled off' from the action of the body fluids, by the protoplasm of a single cell containing it, there is no reason why it should not be carried unharmed wherever this cell goes.

There are in the literature a number of detached observations which corroborate the above findings. Bordet (1914) found that cholera spirilla injected into the blood stream of cholera immune animals may be taken up by the leucocytes before they can be acted on by the circulating lytic bodies. Metchnikoff, Levaditi (1902), Briscoe (1908) and others have shown that red blood cells injected into the previously immunized animal may be phagocytosed before there is time to hæmolyse them.

When, in general practice in India, an abscess appears a few weeks after quinine injection, it is usually not attributed to quinine. The author was once asked to see a case of paratyphoid fever in which an abscess had developed during convalescence at the site of such an injection. The practitioner in charge of the case was certain as to the asepsis of his technique and thought that the interval of a fortnight, which had elapsed since the injection, ruled out the introduction of septic organisms. The former part of this statement is probably true but the latter is not necessarily so. However, since the paratyphoid bacillus is liable to cause suppuration *per se*, the blame was laid at the door of the primary disease and not of quinine. Fletcher (1923) quotes a case to whom two injections of quinine were given, one into each buttock. On one side an abscess developed after a few weeks, but on the other side no ill effects appeared till two months later, when the patient began to feel pain and a large abscess was detected. The natural conclusion to form in such cases as these, is that the infection is carried by the blood stream to the tissues, which have been injured by the quinine.

#### IV. RELATIONSHIP BETWEEN TETANUS AND QUININE INJECTIONS.

##### (a) REPORTS OF OTHER WORKERS.

The possible relationship between tetanus and quinine injections must next be considered. At one time tetanus symptoms were considered as manifestations of quinine poisoning (Sollman, 1906). When it was recognized that true tetanus did occur as a sequel of quinine injection, the explanation why it was more common after quinine injections than, say, after morphine injections was not understood, and questions of this sort were asked in various journals (Query, 1910). Semple (1911), concluded, as the result of animal experiments, that

'(1) when quinine is injected hypodermically or into the muscles, it has a well marked destructive action on the tissues at the site of injection; and in addition to producing these foci of dead tissue which would serve as suitable anaerobic media for the growth of tetanus spores should they by any chance become lodged there, it also gives rise to conditions favourable for infection with "washed tetanus spores" injected into other sites. (2) When quinine is given hypodermically to tetanus infected animals, tetanus germs are transferred from the original site of tetanus infection to the site where quinine has been injected. (3) Pure "washed tetanus spores" given hypodermically to guinea-pigs and monkeys do not produce tetanus; but when quinine is injected hypodermically into another part of the body, either the day before or the day after the spores are given, a large number of these animals contract tetanus. (4) "Pure washed tetanus spores" when mixed with quinine, or weak lactic acid, and injected hypodermically into guinea-pigs invariably produce tetanus, but when mixed with morphia the animals remain well. Quinine and lactic acid when injected hypodermically produce sites favourable for the development of tetanus spores; but morphia and normal saline solutions do not produce this effect. (5) "Pure washed tetanus spores" when injected hypodermically into guinea-pigs remain latent at the site of injection for months, as evidenced by the fact that virulent tetanus bacilli may be recovered from these sites after a period of seven months, and possibly after a much longer period. The importance of this fact in relation to hypodermic injection of quinine is evident. (6) Tetanus infection was present in the intestinal tract of healthy human subjects in four cases out of ten examined. In three of these cases the tetanus bacilli isolated were virulent for guinea-pigs. (7) Some strains of tetanus spores are extremely resistant, and may remain alive and retain their virulence on a rusted nib for as long as 18 years, when the nib is placed in a test tube capped with rubber, and kept in a cupboard at room temperature. (8) Tetanus antitoxine is an efficient prophylactic against tetanus when it is necessary to give quinine hypodermically'.

Semple's work aroused considerable controversy in the press at the time.

Smith (1911) under the title 'quinine without tetanus' wrote, 'in case of a dog bite with a bare possibility of the abrasion having been licked by a rabid dog and a risk of 1 in 10,000 or 1 in 100,000 of hydrophobia, he would feel justified to advise a course of Pasteur's treatment. But with quinine injection the risk of tetanus was not so much as to feel justified, as Semple (1911) says, to advise antitetanic serum. In the first place there is nothing to show that quinine in small doses used in the treatment of malaria (2 grains in 20 min. of water) has any escharotic effect on the tissues, but admitting the slough and the spores what risk does the patient run of developing tetanus? Considerably less than any person with a small boil before the skin has broken'. Stott (1915) said 'granted tetanus may rarely follow the injection of quinine there was no reason in this

why a patient should be deprived of the undoubted clinical value of this method by which he may perhaps quickly escape his infection. Several cases after operation had occurred, but all surgical procedures had not on that account been prohibited'. Palmer (1919) remarks that 'granted few cases of tetanus occurring and these spread over many years, was that in itself a justification for the prohibition of an old method and well tried procedure, unless it could be proved—and up to the hilt—that incidence of this disease was not due to the faulty method of administration?' There were several replies to these criticisms, but the following two are perhaps the most important. The Editor of the *Indian Medical Gazette* (1915a) summed up the situation when he remarked 'until fairly recently it was believed that hypodermic injections of quinine, as of other medical agents, meant more rapid and more concentrated action than is possible by mouth. Close clinical observations cast doubts on this orthodox belief. The expected good results were not obtained from hypodermic injections of quinine. The first result was that the physicians increased the dose of injection from 2-4 grains to 8-10 grains in the hope of getting good results'. Again the Editor (1915b) states 'our feeling is that Sir Semple pointed great dangers, nevertheless hypodermics of quinine have been given thousands of times without ill effects, but there are *other* and safer ways of administering quinine'. Finally the Editor (1925) says 'our view is that in cases of persisting vomiting or failure to absorb the drug by the alimentary canal it may be necessary to give one or two doses by the muscle and to run the slight risk of local necrosis, but such cases are very exceptional. Intramuscular injections must be given with a full sense of responsibility and should be confined to the cases in which they are considered to be the lesser of the two evils'. While Semple (1919) defended himself thus 'I do not think that medical men are in a hurry to publish cases of tetanus following hypodermic quinine. The fact that it is possible to bring about the death of a patient by using a remedy intended to cure is quite sufficient to simulate medical men to leave no stone unturned to avoid calamities of this nature. In words of the late Professor Maclean, there is something revolting in a death brought directly or indirectly by a remedy intended to cure'.

#### (b) DISCUSSION OF TETANUS.

It will have been gathered from the above that tetanus occurring as a sequel of quinine injection was no longer doubted even by the protagonists of parenteral quinine, but the argument which they gave justifying their favouring quinine by this route was its greater efficacy when so administered—a point which has been discussed in full detail earlier in this paper. Under quinine as a cause of suppuration, the work of Rous and Jones has been mentioned. These experiments are also true in respect of tetanus spores. But why should necrosis and suppuration, resulting from quinine injections, specially favour tetanus?

According to Villard (1903) the factors which favour multiplication of tetanus bacilli in the body are:—(1) dirt in the wounds, (2) means which keep leucocytes away from the inoculated spot, (3) means which afford mechanical protection to the bacilli, (4) addition of certain chemicals, *e.g.*, lactic acid, (5) severe mechanical lesions, *e.g.*, fracture, etc., (6) introduction of certain saprophytes. In cases of tetanus following quinine injections, 1st, 5th and 6th factors may be ruled out; but the 2nd, 3rd and 4th factors are satisfied.

That quinine keeps the leucocytes away from the inoculated spot is well known (Binz, 1891). This is brought about in two ways (a) by impairing

phagocytosis (Golgi, 1891); (b) by causing leucopenia (Marshall, 1901; Sollman, 1906). Regarding mechanical protection, the experiments reported earlier in this paper show that a coagulum forms at the seat of quinine injection. This coagulum is formed by a combination of proteids and the precipitate of the alkaloid resulting from its contact with serum. This may not only afford mechanical protection to tetanus spores, but favour their development by diverting phagocytes from the relatively bland spores to the more irritating coagulum (Heddaeus, 1908). Finally quinine is included with lactic acid among the chemical agents that favour tetanus. MacGilchrist (1911) explains this as follows:—quinine combined with proteids is precipitated in the coagulum in an oxidized form [cf. experiments of Rossabach (1911) on the compound formed by the combination of quinine and albumin, and Kerner's (1869-1870) experiments with dihydroxyl-quinine formed by acting on quinine with permanganate of potash]. The interstices of the coagulum produced by quinine provide a favourable fluid anaerobic medium for the growth of tetanus spores. The action of quinine in inhibiting the oxidation of guaiacum (Binz, 1891) in ordinary blood tests, in inhibiting the so-called 'acid fermentation of blood' (Bonn, 1886) and in diminishing the synthesis of hippuric acid in kidney perfusion experiments (Hale-White, 1901) may be similarly explained; oxygen is used up in the quinine albumin compound, and none is available for guaiacum, for the blood or for the nourishment of the renal cells, respectively.

More recent work by Fildes (1929), stressing the importance of too positive Eh (i.e., oxidation—reduction potential) of tissues affording immunity to infection by *B. tetani* is an additional link in the chain of experimental evidence, that the lag (i.e., the period required for the germination of the spores of *B. tetani*), in a suitable medium depends mainly on the time required for this medium to reach a suitable reducing intensity. The greater the reducing intensity of the medium the shorter the lag, until a zone of reducing intensity is reached at which germination is not observed to occur. This zone may be measured and expressed in terms of Eh. At each pH it approximates to the zone of complete reduction of thionin, i.e., at pH 7.0 it approximates to Eh + 0.01 volts. If spores are placed under conditions in which Eh is more positive than this, they do not germinate.

Though Eh may be the main factor, other factors such as pH also come into play. That pH has a marked effect upon the rapidity with which media attain a negative Eh is another factor to be borne in mind. Thus it has been shown by Fildes (1929) that tetanus spores hardly germinated at all on the acid side pH 6.3; thus if the bacteria causing suppuration had the effect of depressing the pH below this, germination would not occur in spite of the adequate Eh. On the other hand alkali producing organism might have the same effect as alkali in culture, and lead to more negative change in Eh and thus more certain germination. Considerations of this sort may account for the anomalous behaviour of quinine injections causing tetanus. The line of demarcation is too narrow. That quinine, on account of its reducing properties,

is potentially capable of approximating the critical Eh of tetanus spores to that of tissues, thereby affording favourable conditions for the growth of bacteria, will not be denied by any reasonable person.

It will now be clear why morphine or saline injections do not favour the occurrence of tetanus, and a small boil before the skin has broken is less favourable site for the development of tetanus than the lesions produced by quinine injections. The Eh of the subcutaneous tissue fluids is more positive than the Eh required for germination of spores of *B. tetani*, and neither the injections of morphine nor saline, nor the presence of staphylococci or streptococci in the boil, have any property of reducing Eh to the negative critical Eh of tetanus, while quinine injection has.

The present-day opinion is veering away from the nidus theory of tetanus. Thus Bullock and Cramer (1919) have shown that experimental evidence does not support the view that the development of tetanus is influenced by such factors as interference with the circulation, the presence of large masses of dead and lacerated muscle, the presence of cloth, dirt, or foreign bodies generally. Even cutting the femoral artery and injecting a suspension of *B. welchii* into the leg or into the hæmatoma, or injecting suspension of *B. welchii*, together with staphylococci, streptococci, *B. coli*, *B. proteus* or *B. sporogenes*, did not bring about gas gangrene. With the disappearance of the nidus theory, our old conception regarding the ætiology of tetanus should also disappear.

With the accumulation of experimental evidence in favour of the accessory factor producing 'kataphylaxis' or 'defence rupture' or 'negative critical Eh' of tetanus' being a chemical constituent, it is only right that we should express our ideas regarding 'quinine and tetanus' in the light of recent advances. Bullock and Cramer (1919) conclude that 'there can be little doubt that the presence of certain simple chemical constituents of the soil which have the property of producing the kataphylactic phenomena are responsible for the occurrence of gas gangrene and of tetanus'. The general depression of vitality due to malaria may reasonably be assumed to involve also the processes of lysis and phagocytosis which constitute the defence mechanism against infective bacteria, and thus account for the development of tetanus after quinine injection.

It is quite true as Semple (1919) has said that no medical man will be in a hurry to publish his tetanus cases following quinine injections. Whenever a case of this nature comes to light in India, the doctor often denies having given the injection or even having treated the case. About two years ago a case of this nature was reported in the lay press, in which a criminal action was brought against the doctor alleged to have given the injection. The defence was a denial of having treated the deceased, and the medical evidence was that tetanus often followed quinine injections, even in cases where all necessary precautions had been taken.

## (c) CONCLUSIONS.

Evidence has been adduced above to show that injections of quinine intramuscularly create a favourable site for the development of tetanus, and that following them cases of tetanus have been reported even when all reasonable precautions were taken. It is known both from experimental and clinical evidence that quinine administered orally is quite as efficacious as that given by parenteral injection in the vast majority of cases. It would therefore appear that the use of quinine injections (unless in exceptional cases) amounts almost to malpraxis in a country like India (a) where dysenteric ulcers are not uncommon, (b) where tetanus spores are known to inhabit the intestine (Semple, 1911), (c) when the 'walling off' property of phagocytes has been established as a fact (Rous and Jones, 1916), (d) when transportation of tetanus germs from one part of body to another part, where quinine is injected, has been demonstrated beyond doubt (Semple, 1911; Vincent, 1904), (e) when the oxygen depletion properties of quinine have been confirmed up to the hilt (Binz, 1891; Bonn, 1886; Hale-White, 1901), (f) when genuine cases of tetanus following quinine injection have been reported off and on (Semple, 1911, 10 cases; Stott, 1915, one case; Clyne, 1917, one case), and (g) when oral quinine has been ascertained by experimental and clinical evidence to be as efficacious as parenteral quinine.

## V. INDICATIONS FOR INTRAVENOUS INJECTIONS OF QUININE.

In pernicious cases where it is impossible to administer quinine by mouth, it should be injected by intravenous route. Our ideas regarding dilution of quinine by this route, since MacGilchrist (1911) suggested a dilution of 7 grains of quinine in 2-3 pints of saline, have undergone considerable changes. We now know as pointed out by Thomson (1917) that great dilution can be obtained in the circulating blood by attention to technique, even when comparatively strong solutions are used for injections, *i.e.*, 9-15 grains of quinine in 5-6 c.c. of saline. If 20 seconds are occupied in injecting one c.c. and a pause of some seconds made between each subsequent c.c., it is not necessary to use a dilution of pints. Knowles (1918) suggests quinine acid-hydrobromide as the best salt for intravenous injection. After citing 28 cases and 139 intravenous injections of quinine acid-hydrobromide he concludes that this 'salt of quinine in 10 c.c. doses of 5 per cent solution by the intravenous route appears to be a perfectly safe method, and is infinitely preferable to intramuscular injection from every point of view'. He lays stress on injection being given very slowly and the patient kept lying down for 15 minutes after injection. He sterilizes the solution in an autoclave at 110°C. for 10 minutes.

## VI. INDICATIONS FOR INTRAMUSCULAR INJECTIONS OF QUININE.

There are however times when even the intravenous route is unsuitable or impossible, *e.g.*, small children and obese persons with coma. In such cases

parenteral quinine is justifiable. No one with any experience in treatment of malaria is likely to deny its value in these cases. In my opinion, it is as unjustifiable to withhold injections in such cases when the patient's life is in danger, as it is to employ quinine injections in ordinary cases of malaria when the patient can take quinine orally.

## VII. CHEMICAL CHANGES IN QUININE SOLUTIONS.

Before I conclude this paper I wish to emphasize two other points. The first point is regarding deterioration of quinine on boiling. There is an impression in some quarters that practically all quinine salts become decomposed when boiled. Thus according to Fluckinger (1894), 'quinine preparations when exposed to heat and sunlight lose water of crystallization and become decomposed, a yellowish brown product being formed which he names "quineritin". In some cases boiling produces this change in a few minutes'. MacGilchrist (1911) states 'practically all of the quinine preparations, when exposed to sunlight, acquire—whether in solid form or in solution—a yellowish brown colour. Heat seems to accelerate this change in quinine; and boiling whether for purposes of sterilization or of hastening solution, produces this change in some of the quinine preparations in a few minutes'. Semple (1911) remarks 'it is a well known fact that boiling produces deleterious changes in some salts of quinine in a few minutes'.

### EXPERIMENTS.

With a view to finding out what percentage of quinine base was lost from quinine acid hydrobromide solutions during boiling, experiments were carried out in this laboratory by the author. Solutions of quinine acid-hydrobromide were made in water (10 grains in 10 c.c.), according to the strength usually employed for parenteral injections. Four solutions were made and labelled 1, 2, 3 and 4. Nos. 1, 2 and 3 were boiled for 3, 5 and 10 minutes respectively, while No. 4 was kept as a control. Estimations of quinine from these solutions were made gravimetrically as well as nephelometrically, controlled by solution No. 4. The following were the results:—

Number of solution.	Strength of solution.	Period of boiling in minutes.	Quinine estimated gravimetrically.	Quinine estimated by Sinton's method.
1	Grains 10 in 10 c.c. of water.	3	9.56 grains	10 grains.
2	Grains 20 in 20 c.c. of water.	5	19.29 grains	20 grains.
3	Do.	10	19.52 grains	20 grains.
4 (control).	Do	..	..	20 grains.



It will be seen from the above that practically no quinine was lost, and the infinitesimal loss indicated gravimetrically may have been due to errors of technique. No change in the colour of the boiled quinine solutions was noticed.

### VIII. GROWTH OF MICRO-ORGANISMS IN QUININE SOLUTIONS.

The next point is regarding the inimical properties of quinine towards fungi and bacteria. In the literature on the subject solutions of quinine sulphate alone are spoken of as being prone to become infected with moulds (*Pencillium*), and that also on exposure to air (Marshall, 1901). But experiments performed in the Edinburgh University laboratory showed that the growth of moulds was not limited to the solutions of quinine sulphate, and the genus of fungus was not confined to *Pencillium*. The solutions of various quinine salts were kept in stoppered bottles, and even then growths were present in solutions of quinine quinate, acid-hydrobromide, acid-hydrochloride, bisulphate, urea acid-hydrochloride, lactate and arsenite. The genus *Mucor*, as judged from zygospore formation, was present in one or two instances, but the PERISPORIACEÆ predominated. Both genera, *Aspergillus* and *Pencillium* with their respective gonidiophores were in evidence, the former being almost as common as the latter. *Perithecia*, indicating the less frequent mode of reproduction, were observed in solutions of acid-hydrobromide, lactate and arsenite. It would appear therefore that quinine is not so inimical to the higher members of fungi as to the lower yeast forms, which are said to be as susceptible to its action as bacteria are (MacGilechrist, 1911). The procedure adopted by some practitioners in large towns, of storing quinine solutions in sterile rubber-capped bottles and drawing it off as required into a syringe, is therefore open to serious criticism.

### IX. CONCLUSIONS.

(1) Quinine administered orally along with alkaline mixture, as advocated by Sinton (1923, 1930), if it be retained, is as efficacious therapeutically as quinine administered by injection.

(2) Parenteral injections of quinine are very liable to produce local necrosis and suppuration of the tissues.

(3) The lesions resulting from quinine injections afford favourable sites for the development of tetanus spores. This is specially the case with intramuscular injections, because of the presence of lactic acid in the muscular tissues and, if suppuration occurs, of the presence of deep seated pus which forms an ideal anaerobic medium for the development of tetanus. In the case of subcutaneous injections the Eh of the tissue fluids is less suitable for the germination of tetanus spores, and suppuration is more likely to be followed by the formation of an open sore, in which the occurrence of anaerobic conditions is less probable.

(4) When oral administration of quinine is impracticable, as in comatose pernicious cases of malaria, quinine should be given intravenously wherever possible. This may not be feasible under two conditions, namely, in small children and in very obese patients where the veins are not prominent. Under these two conditions *only* should parenteral injections be resorted to, the subcutaneous route being preferable to the intramuscular one. In giving subcutaneous injections, care should be taken to see that the point of the needle has completely pierced the skin, as intradermal injection will almost certainly produce an open ulcer.

(5) For parenteral injection, quinine should be given in dilute solution, *e.g.*, 5 grains in 10 c.c. water or saline. Such dilute solutions cause less necrosis of the tissues than more concentrated solutions. The more dilute solutions may perhaps cause more widespread œdema, but this subsides quickly. Subcutaneous injections should be given slowly. *Quinine acid-hydrobromide or hydrobromate is the salt of choice for subcutaneous injections.*

(6) Quinine solutions for injection should be freshly prepared and should not be stored in rubber-capped bottles. The sterility of the solution should be ensured by boiling for at least 3 minutes. Solutions of quinine acid-hydrobromide remain unaltered after boiling for 10 minutes. The syringe should be sterilized by boiling, and not merely rinsed with spirit or alcohol as is done by many practitioners.

(7) The administration of quinine by injection is a responsible procedure and should always be undertaken by the medical officer himself, and never relegated to any member of his subordinate staff.

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NOTE ON SOME UNSUCCESSFUL ATTEMPTS TO INFECT A  
MONKEY BY INJECTIONS OF LIVING  
MALARIAL SPOROZOITES.

BY

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MANY workers have made unsuccessful attempts in the past to infect different species of monkey with human malarial parasites. Mesnil and Roubaud (1917, 1920), however, claim that they succeeded in infecting a chimpanzee with *P. vivax*, but there is considerable doubt about the validity of this claim (Thomson and Robertson, 1929; Craig, 1931).

The majority of these experiments have been carried out by the injection of blood containing human malarial parasites. In the present research malarial sporozoites were used.

Green (1932) injected approximately 40,000 sporozoites intramuscularly in a young monkey, *Silenus irus* (*Macacus cynomolgus*). These sporozoites were taken from six pairs of salivary glands from mosquitoes infected by feeding on human cases of malignant tertian malaria (*P. falciparum*) 17 days previously. Five days later another injection of about 15,000 sporozoites was made into the same animal.

Subsequent observations, carried out daily over a period of thirty-two days on this monkey, revealed no febrile reaction, no enlargement of the spleen and no parasites in the blood.

Green (1932) concluded that either this monkey was insusceptible to infection with the sporozoites of *P. falciparum*, or that the sporozoites were non-viable at the time of injection; or again that the intramuscular route was unsuitable. The possibility that the monkey was refractory to any form of malarial infection was ruled out, as the animal later proved capable of infection with *P. inui* at the end of the observation period. The latter infection was followed by a typical attack of the disease. Daily observations over 10 months showed no signs of *P. falciparum*, although *P. inui* was constantly present.

In Green's experiment two hours elapsed between the dissection of the mosquitoes and the injection of the sporozoites. In the experiments recorded

below only 10 minutes elapsed before injection, thus the chances of any lowered vitality of the parasites were greatly diminished. The subcutaneous and intraperitoneal routes were used for injection, not the intramuscular one.

The sporozoites were obtained from the glands of three different specimens of *Anopheles culicifacies*, found infected in nature from the Karnal District, Punjab. The infections were presumably of human origin.

## EXPERIMENTS.

### *Experiment No. 1.*

On 5th September, 1932, the salivary glands of a specimen of *A. culicifacies* dissected in normal saline, were found to show a heavy infection with active sporozoites. These sporozoites were collected in normal saline solution and injected subcutaneously into a very young specimen of *Silenus rhesus* (*Macacus rhesus*), the common brown monkey of Northern India. Blood examinations, by both the thin and thick film methods, were made daily for 8 days with negative results.

### *Experiment No. 2.*

On 14th September, the same monkey was again injected subcutaneously with sporozoites from another naturally infected *A. culicifacies*. Blood examinations were made daily for another 10 days and then weekly for a period of 7 weeks. During this time the monkey showed no abnormal symptoms and all the blood examinations revealed no malarial parasites.

### *Experiment No. 3.*

On 10th November the same monkey was again injected intraperitoneally with sporozoites from another heavily infected specimen of *A. culicifacies*. Daily blood examinations were made for ten days and then weekly examinations for another 3 weeks. These examinations were all negative and the monkey showed no signs of illness.

## SUMMARY.

A very young specimen of *S. rhesus* received two subcutaneous and one intraperitoneal injection of living malarial sporozoites from the salivary glands of three specimens of *A. culicifacies* found infected in nature. Very numerous blood examinations were continued for a period of about 100 days after the first injection and 40 days after the last one. These examinations were made by both the thin and thick film methods and no malarial parasites were found. The monkey showed no signs of illness at any time although carefully observed, was still healthy more than 5½ months after the primary inoculation, and blood examination revealed no parasites.

My thanks are due to Laboratory Assistant Ambrose David for his assistance in these experiments.



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FURTHER OBSERVATIONS ON A MALARIA SURVEY IN  
THE JORHAT DISTRICT, ASSAM, WITH SOME NOTES  
ON THE ANTI-MALARIA MEASURES  
EMPLOYED.

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INTRODUCTION.

THE preliminary data of a malaria survey carried out in this district from 5th July till 31st December, 1930, have already been published by one of us (Manson, 1931). This survey was continued during the year 1931, and the principal results of a research carried out over a period of one complete year (1st August, 1930, till 31st July, 1931) were published as a joint paper (Manson and Ramsay, 1932).

The main aim in the above two papers was to record the various species of Anopheline mosquitoes which are found breeding in the Jorhat District, to prove the carrier species and to present a clear picture of their ecology and especially of *A. minimus* which was found by dissection to be the only species transmitting malaria.

The work recorded in the present paper deals mainly with the second completed year, beginning on 1st August, 1931, and ending on 31st July, 1932. In this publication we are recording the additional species which were found, with a note on their bionomics, also further notes on the species already described.

The findings in the infectivity survey and the anti-malarial measures adopted, both temporary and permanent, are also recorded.

### MOSQUITO FINDINGS.

From 5th July, 1930, until 31st July, 1931, the following species of Anopheline mosquitoes were identified either in their adult or larval stage in the Jorhat District :—

- |                               |                              |
|-------------------------------|------------------------------|
| 1. <i>A. minimus</i> .        | 9. <i>A. culicifacies</i> .  |
| 2. <i>A. vagus</i> .          | 10. <i>A. maculatus</i> .    |
| 3. <i>A. hyrcanus</i> .       | 11. <i>A. karwari</i> .      |
| 4. <i>A. philippinensis</i> . | 12. <i>A. aitkenii</i> .     |
| 5. <i>A. aconitus</i> .       | 13. <i>A. tessellatus</i> .  |
| 6. <i>A. kochi</i> .          | 14. <i>A. gigas</i> .        |
| 7. <i>A. fuliginosus</i> .    | 15. <i>A. jeyporiensis</i> . |
| 8. <i>A. barbirostris</i> .   | 16. <i>A. leucosphyrus</i> . |

In addition to the above species we have now added the following :—

- |                       |     |                          |
|-----------------------|-----|--------------------------|
| 17. <i>A. ramsayi</i> | and | 18. <i>A. umbrosus</i> . |
|-----------------------|-----|--------------------------|

#### *A. ramsayi*.

This species was found first on 26th December, 1931, and was evidently only a transient visitor, as we were unable to find it again after the 17th March, 1932.

The type of breeding ground favoured was clear water in or near rice-fields. An analysis showed that the water contained only a very low saline content; the actual percentage found being 0.0088. This is in marked contrast to *A. umbrosus* which generally breeds in water with a decidedly higher saline content.

#### *A. umbrosus*.

This species was first found in this district on 4th October, 1931. It appears to be widely distributed over the whole area surveyed.

#### Nature of breeding grounds of *A. umbrosus*.

*A. umbrosus* larvæ were found in stagnant water in ditches, hullahs and pools, exposed to sunlight or in light shade, and also in denser shade than any other species in Assam; but so far not in places shaded by thickly matted vegetation.

Although a very thorough search was repeatedly carried out in densely shaded swamps and hullahs, on no occasions were *A. umbrosus* larvæ found in breeding places covered with really dense shade. The surface of the water in *A. umbrosus* breeding places was often partially covered with a mass of dead leaves and vegetable detritus with a bed which was peaty in composition.

The importance of a close study of this species is evident. Vegetation which provides dense shade over watercourses (streams and swamps) is greatly employed in Assam as an anti-larval measure.

Were *A. umbrosus* an important vector of malaria in Assam, it would obviously be a great danger to introduce a type of shade in an anti-larval campaign which would be suitable for the species.

#### Bionomics of *A. umbrosus*.

In view of the fact that this species has been incriminated as a malarial vector in the Federated Malay States, it was considered necessary to make a thorough study of its bionomics. The adult mosquito attacks human beings ferociously in the jungle during the day-time, and is a voracious feeder, but we were unable to collect any adult specimens in the jungle at night-time. A very careful search was also made in the coolie lines of a garden where the lines were within half a mile of several *A. umbrosus* breeding areas. Many nights were spent on this search, but no *A. umbrosus* adults were found in human habitations or in cow-sheds.

This seems to point to the fact that *A. umbrosus* is probably not engaged in the transmission of malaria in this district, but has normally some source other than human for its blood meals. To determine this more fully, two further experiments were carried out. An area was chosen in a small clearance in a local forest where Nepali sawyers were engaged in cutting timber. These people were housed within fifty yards from *A. umbrosus* breeding places. Several of the men suffered from malaria, and most of them had enlarged spleens. In addition to *A. umbrosus*, other species such as *A. minimus*, *A. philippinensis*, *A. hyrcanus* and *A. barbirostris*, were breeding freely in the cleared and partially cleared areas. Traps consisting of tea baskets lined with cow dung and covered with black cloth were placed in the Nepali houses. A cow was tethered in a convenient shed and, as monkeys were very prevalent, it was thought possible that *A. umbrosus* might feed on monkeys. Accordingly a monkey (*Macacus rhesus*) was provided with a separate house in the same compound. The results were very interesting. The only species caught in human habitations were *A. minimus* and *A. philippinensis* (both species had ingested blood). In the monkey house and cow shed *A. hyrcanus*, *A. barbirostris* and *A. philippinensis* were found and all had ingested blood. On no occasion was *A. umbrosus* found.

This investigation was started about the beginning of November, but as the night temperature dropped about a fortnight afterwards, below 60°F., the result could not be called conclusive. A further experiment on the same lines is being carried out during the current year at an earlier date, and the results will be recorded in due course.

In the second instance, a small group of houses was selected within one hundred yards of a breeding area of *A. umbrosus*. This group of houses is

outside the area where anti-larval measures are being carried out and breeding places of *A. minimus* were also very plentiful in this area. A careful search was made nightly for six weeks in the houses for *A. umbrosus* but none was found, whereas *A. minimus* were found in large numbers until the night temperature dropped below 60°F. All the inhabitants of the houses had suffered from malaria and all had enlarged spleens. This experiment was also terminated as the night temperature fell below the range which is suitable in Assam for adult Anophelines feeding in unheated human habitations.

So far as we can judge from the above investigations it would appear that *A. umbrosus* does not normally feed on human blood in Assam.

In a degree of shade such as is suitable for *A. umbrosus*, we frequently find other species such as *A. aithenii*, *A. leucosphyrus*, *A. hyrcanus* and *A. barbirostris*, but a degree of shade which is suitable to these species is inimical to *A. minimus*. The degree of shade and methods of application, however, which are being applied in Assam to eliminate *A. minimus* will also eliminate *A. umbrosus*. Hedges providing dense shade are only planted over streams and drains where there is sufficient flow of water to keep the channel from becoming silted up. The degree of shade furnished is sufficient to prevent vegetation, grass, or weeds from growing in these channels, and thereby eliminates all Anopheline species, as owing to the absence of vegetation, larvæ are unable to find anchorage for their tail hooks. Further *A. umbrosus* has poorly developed tail hooks, also rudimentary palmate hairs and is readily eliminated by velocity of current.

During the present season, further experiments are being carried out to determine completely the bionomics of *A. umbrosus*, and particularly to determine what type of blood this species favours. *A. umbrosus* disappeared on the 23rd December, 1931, and in spite of a very careful search, no larvæ were found again until 9th May, 1932.

Experiments were carried out in the laboratory to determine the length of time this species can survive in its larval form during the cold weather. Fully developed larvæ were caught and placed in a porcelain dish in their natural water on the 19th December, 1931. The last larva died on 22nd January, 1932. This gives a total length of life of 34 days in partly artificial conditions in larvæ already fully grown. There was no pupation in this experiment. It is evident from this experiment that *A. umbrosus* larvæ can winter for at least two months.

#### *A. tessellatus.*

The larvæ of this species were first found on 24th April, 1932, and an adult specimen was caught on 17th March, 1932. The larvæ were collected from a tank with rather muddy water. *A. tessellatus* either in its larval or adult form is relatively uncommon in Assam, but a larva was again found on 25th June, 1932.

*A. leucosphyrus.*

Adults of this species were obtained from two tea estates widely apart. The larvæ were found on 31st October, 1931, and generally in the same areas as *A. umbrosus*. This species occurred in larval form on several estates and always in lightly shaded areas. In 1932 the larvæ were first found on 13th February.

*A. gigas.*

*A. gigas* was found plentifully in larval form from 12th January, 1932, until 6th March, 1932. It generally bred in permanent waters such as the Dessoï River, also in clear pools. This species seems to disappear from this district annually about the end of April.

*A. aitkenii* var. *bengalensis*.

This variation of *A. aitkenii* was found on 4th April, 1932, and the larvæ occurred in pools in the Dessoï River bed but not in shade.

*A. culicifacies.*

The larvæ were found in the Dessoï River in sandy pools with relatively clear water and little vegetation. Very few adults were collected, although a careful search was specially carried out owing to the fact that this species is a well known vector of malaria in other parts of India.

*A. maculatus.*

*A. maculatus* occurs plentifully in the hilly districts, but is relatively scarce in the plains of the Jorhat area. Larvæ were found in the river beds in clear pools during March and April, but were very rare.

*A. karwari.*

This species was found in fairly large numbers in its larval form and was first observed on 22nd February, 1932. It was generally found in seepages, sometimes on low-lying land in clear stagnant water with grassy edges. Adults were seldom found.

## SOME PARASITES OF ANOPHELINE MOSQUITOES.

## (a) PARASITES OF LARVÆ.

*Vorticella* was a very common ectoparasite but did not seem to influence the activities of larvæ unduly. This protozoon was found on most of the species of Anopheline larvæ in this district. Occasionally heavy infections with ciliated protozoa were found and seemed to be present during the shedding of the larval skins between successive instars. *Hydrachnid* mites were also common ectoparasites in the larval stage and several varieties were found.

## (b) PARASITES OF ADULTS.

In addition to the larval form of the helminth *Agamomermis* several gut infections with *Filaria* were found during the year and again several heavy infections with *Trypanosomes*. Mites were abundant on some species, especially *A. vagus*. No *Culicoides anophelis* were found in the year under review.

## INFECTIVITY SURVEY.

The infectivity survey was continued, but as anti-larval measures were directed against *A. minimus* breeding places, we found great difficulty in collecting adults of this species in the controlled areas. The number of each species dissected during the year, and the malarial findings are recorded in Table I,

TABLE I.

*Anopheline mosquitoes dissected from 1st August, 1931, to 31st July, 1932.*

Species.	Aug. 1931.	Sept. 1931.	Oct. 1931.	Nov. 1931.	Dec. 1931.	Jan. 1932.	Feb. 1932.	Mar. 1932.	April 1932.	May 1932.	June 1932.	July 1932.	Total.
<i>A. minimus.</i>													
Number dissected ..	1	12	36	203	45	1	0	0	1	69	48	102	518
Number infected ..	0	1s	1s	2s 1g	0	0	0	0	0	3s 3g	0	2s 2g	9s : 6g.
<i>A. philippinensis.</i>													
Number dissected ..	48	49	33	62	71	56	43	13	32	12	19	48	496
Number infected ..	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>A. fuliginosus.</i>													
Number dissected ..	4	5	5	2	2	0	0	6	63	88	82	38	295
Number infected ..	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>A. kochi.</i>													
Number dissected ..	11	17	20	62	11	10	20	15	8	22	58	12	266
Number infected ..	0	1g	0	0	0	0	0	0	0	0	0	0	1g
<i>A. hyrcanus.</i>													
Number dissected ..	101	88	82	236	144	245	170	199	184	115	243	200	2,007
Number infected ..	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>A. vagus.</i>													
Number dissected ..	240	209	103	24	9	3	3	1	49	353	529	336	1,859
Number infected ..	0	0	0	0	0	0	0	0	0	0	0	0	0

*Note.*—The symbols 's' and 'g' stand for 'salivary glands' and 'gut' respectively, and the numbers attached denote the number of insects in which these were found infected.



TABLE I—concl'd.

Species.	Aug. 1931.	Sept. 1931.	Oct. 1931.	Nov. 1931.	Dec. 1931.	Jan. 1932.	Feb. 1932.	Mar. 1932.	April 1932.	May 1932.	June 1932.	July 1932.	Total.
<i>A. barbirostris.</i>													
Number dissected ..	1	7	4	5	2	6	7	1	8	18	23	4	86
Number infected ..	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>A. karwari.</i>													
Number dissected ..	0	1	3	1	0	0	0	0	0	0	0	1	6
Number infected ..	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>A. aconitus.</i>													
Number dissected ..	0	0	3	19	39	40	14	17	19	11	7	5	174
Number infected ..	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>A. umbrosus.</i>													
Number dissected ..	0	0	0	3	0	0	0	0	0	0	0	0	3
Number infected ..	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>A. culicifacies.</i>													
Number dissected ..	0	0	0	0	0	0	0	0	1	3	1	0	5
Number infected ..	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>A. maculatus.</i>													
Number dissected ..	0	0	0	0	0	0	0	0	0	1	1	0	2
Number infected ..	0	0	0	0	0	0	0	0	0	0	0	0	0

while Table II gives the results of blood slides examined. The importance of *A. minimus* as the vector of malaria in Assam is again clearly shown. In

TABLE II.

Blood slides examined from fever cases from 1st August, 1930, to 1st July, 1932.

Number of slides examined.	Number of positive slides.	Number of negative slides.	INFECTIONS.					
			M. T.	B. T.	Q. T.	M. T. and B. T.	B. T. and Q. T.	M. T. and Q. T.
1,803	800	1,003	632	120	8	36	2	1
			79%	15%	1%	4.5%	0.25%	0.12%

September a gut infection was found in *A. kochi*, but this species does not appear to be of any sanitary importance in the Province, as it feeds mainly in cow-sheds.

In Graph I are recorded the maximum and minimum temperatures, the vapour pressure or absolute humidity, the rainfall and the period during which mosquitoes were found in nature to be infected with malaria; while Graph II shows the relationship of the monthly means of absolute humidity and minimum temperatures to fever rates.

### ANTI-LARVAL WORK.

This was started on 21st March, 1931, and was carried out without interruption until the middle of November, when the night temperature had already fallen below 60°F. In 1932 anti-larval work started on 15th March when the night temperature rose to 60°F., and will be continued until the night temperature again falls below 60°F., in November 1932.

From the infectivity rate of *A. minimus* already recorded in previous publications, it is evident that there is a high degree of infection during November, and the finding of gut infection in *A. minimus* early in April shows that anti-larval work should begin not later than the middle of March in each year.

### PERSONNEL AND ROUTINE.

On each tea garden a fully trained Malarial Surveyor was put in charge of the anti-larval operations and was provided with an anti-malarial squad of four to six coolies who worked under his supervision. The hours of work observed were from 8 to 12 in the morning, and from 2 to 4 in the afternoon daily. The policy pursued was that anti-larval work was carried out in the forenoon, and the other areas not dealt with that day were surveyed in the afternoon, the larval catches being sent to the Central Laboratory for identification. Whenever possible an independent surveyor went over each area to check the work of the permanent surveyor. This system means that control measures and an anti-larval check go on simultaneously, and an absolute check can be made on the results obtained.

A register showing all areas investigated and treated is maintained and a copy is sent daily to the Research Laboratory.

The form used is as follows :—

*Daily report of anti-malarial work for*

Name of gardens.	Areas searched to-day.	Number of <i>A. minimus</i> areas found to-day.	ANTI-MALARIAL MEASURES ADOPTED.		
			Areas oiled.	Areas treated with Paris green.	Areas treated biologically.

A control board provided with counters of different colours is kept on each garden. The counters are hung on numbered pegs, corresponding to the numbers on the control maps. These counters show the dates of treatment and the colours show the methods adopted (oil, Paris green or biological).

### ANTI-LARVAL MEASURES USED.

In the Jorhat area the measures adopted have been (i) temporary and (ii) permanent.

#### TEMPORARY MEASURES.

##### *Oiling.*

During the earlier part of 1931 a mixture of crude oil and kerosene oil was used but this was replaced for various reasons. Kerosene oil unfortunately is a readily saleable commodity, and in spite of constant vigilance, a certain amount which should be used for the legitimate purpose of control work is lost.

It is therefore better to use a mixed oil which presents less temptation to the coolie and which also has valuable properties lacking in the original crude oil and kerosene mixture. The type of oil selected was 'Malariol' which was supplied by the Burmah Oil Company, Digboi, Upper Assam, at a cost of annas seven and pies six per gallon. The cost has now been reduced to annas six per gallon. This oil is very effective, spreads very readily in a thin even film, is highly toxic to larvæ, and, in addition, possesses the valuable property of 'burning up' all vegetation and grass along the banks of drains, or streams, amongst which larvæ shelter. In experiments in the laboratory and also in the field, it is found that the oil destroys all larvæ, Anopheline or Culicine, within fifteen minutes. This is of the utmost importance in Assam where rainfall is irregular and heavy and where immediate action by larvicides is necessary for success.

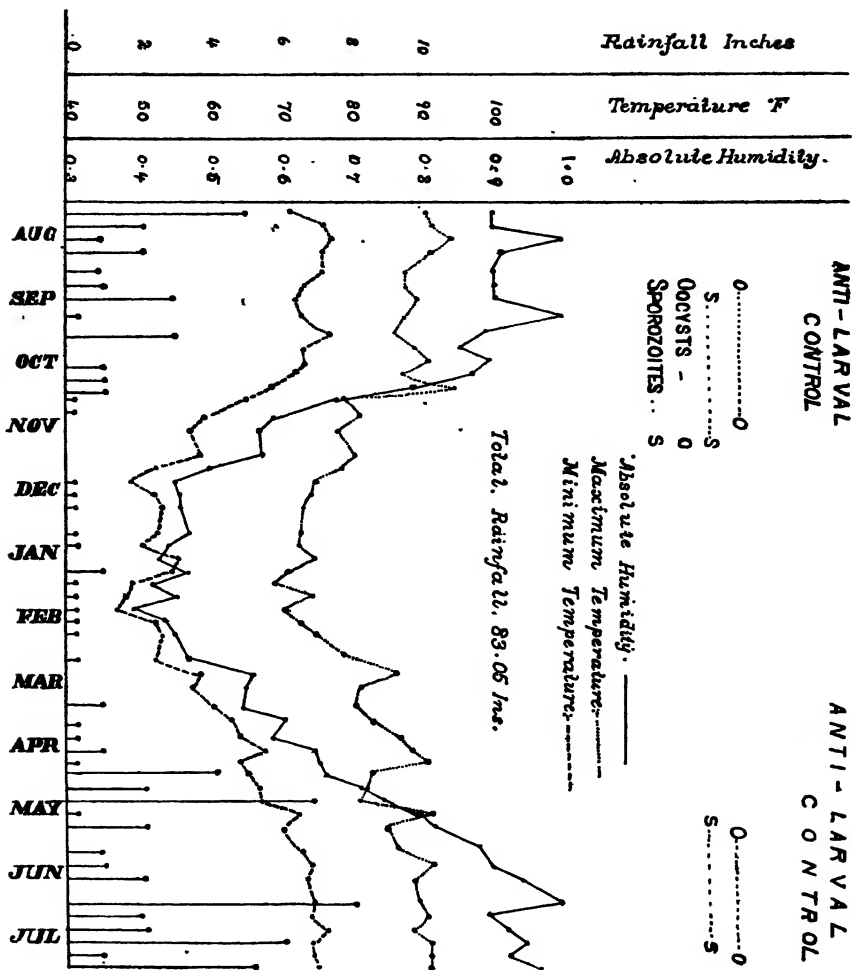
The great advantage of oil over Paris green is that it destroys Culicine as well as Anopheline larvæ, and thus removes the 'general mosquito nuisance'. This is greatly appreciated by the entire community.

The type of oil sprayers used were the 'LADYWOOD' Knapsack Oil Sprayer, and the 'VERMOREL ECLAIR' Knapsack Oil Sprayer. The latter type was found to be the most efficient in use.

*Amount of oil required.*—One gallon of 'Malariol' will suffice to spray efficiently about 250 lineal yards of one edge of the stream. When spraying streams or drains, the banks only should be sprayed where the water touches the sides. It is not necessary to oil the centre of the channel where there is flowing water, as larvæ breed only at the edges of streams, drains, pools or tanks, unless where there are islands of vegetation in the course of the channel, or in pools or tanks.

GRAPH I.

# SEASON 1931-32.



We have found drip-cans ineffective, as the oil is carried down the centre of the channel where the rate of flow is greatest, and frequently does not touch the edges where larvæ are breeding.

### *Paris green.*

The sphere of action of this larvicide in Assam is, in our opinion, limited to the treatment of more or less stagnant water in pools, swamps or seepages where the film is not readily disturbed. Its action is much slower than that of oil, and little larvicidal destruction occurs in less than three hours. It has no action on very young larvæ, which are unable to ingest the relatively large particles of Paris green. These larvæ, however, are destroyed by the application of Paris green during the following week. Paris green does not prevent egg deposition and this method has the advantage of acting as a trap, as gravid females do not seek more distant breeding grounds. This advantage is more than counterbalanced by the disadvantage that they do not seek more distant breeding grounds, and having gone far distant they are unable to return.

The great disadvantage of Paris green in Assam is the heavy rainfall which seriously disturbs the surface film. If heavy rain falls within three hours after dusting, it nullifies to a large extent the work done, and the process of dusting should be immediately repeated.

Paris green does not destroy vegetation and can safely be used where efforts are being made to establish dense shade. It can be used effectively in extensive undrainable swamps which cannot be controlled by oiling.

*Dilutions and diluents.*—The usual dilutions recommended are 1 to 2 per cent, but we have found that a 5 per cent mixture is more efficient and more rapid in action. Finely powdered soapstone or softstone is the best diluent, but 'soorki' (brick dust) and road dust are useful where hand throwing can be carried out.

*Apparatus.*—A mixer is essential for securing an evenly distributed powder. We have used various types of blowers but find the '*Peerless rotary dust-gun*' is the most efficient and economical in use, as one man can manipulate one machine.

*Effects on fish, crops, etc.*—It has no lethal action on fish, and does not, therefore, preclude their employment for anti-larval work. It is harmless to crops, animals and humans in the percentage used. We usually apply a mixture of 1 lb. Paris green and 19 lbs. soapstone or softstone powder per acre.

### PERMANENT MEASURES.

Permanent measures have included the filling in and levelling of broken surfaces (pools, borrow-pits, etc.), draining of suitable small areas where this could be done cheaply and effectively, and the establishment of dense shade over drains and natural watercourses.

*For narrow drains.*

*Duranta hedges* have been found very suitable, but to obtain sufficient shade, at least four rows of cuttings should be planted on both sides of the channel. *Duranta* can easily be established in 2 years to give sufficient shade. The planting should be done in the early part of the monsoon season to ensure that a due proportion of cuttings will take root.

Well matured cattle manure, or better still, sulphate of ammonia, is required for impoverished soils. Sulphate of ammonia in the proportion of 2 ounces per square yard along the area planted is sufficient to induce a response in backward plants in 3 to 4 weeks' time. Cattle manure takes much longer and is much more inconvenient to apply. Young shoots should be kept moist during hot dry spells with a covering of thatch.

*Duranta* may be grown from seed in Assam, but the growth is very slow, and young plants under six months are not suitable for replanting. Planting up both sides of a drain or ditch with *Duranta* cuttings, 4 or 5 rows deep, costs about Rs. 40 per mile.

*For swamps (hullahs and jans).*

'Tarapat' is one of the normal flora of swamps in Assam, and this growth should be encouraged and assisted by filling in vacant areas. Planting at a distance of one yard apart is sufficient as these plants spread rapidly. When transplanting, care should be taken to leave one or two leaves untouched so as to provide suitable respiration to the plant. If this is not done results are often disappointing. The cost of planting up Tarapat in swamps is about Rs. 4 per acre, the price varying a little according to the distance the Tarapat has to be transported. This applies to planting Tarapat 3 feet  $\times$  3 feet.

*Eugenia Balsamea*.—This plant is also useful for shading, and grows on slightly raised turf, just above the water level. It is evergreen, and is not eaten by cattle.

*Hibiscus*.—In areas protected from cattle trespass, this is a valuable substitute for *Duranta*. It is much more rapid in growth and provides dense evergreen shade.

*For wide jans and river courses.*

Rain trees (*Pithecolobium Saman*) and Soom trees (*Machilus bombycina* King) are very rapid in growth and provide excellent shade over channels which are too wide to cover with *Duranta* hedges. Bajhal (*Pseudocostachyum polymorphum* Munro) and bamboos also provide sufficient shade, but Jack-fruit trees (*Artocarpus integrifolia*) have the disadvantage that the fruit is readily eaten by tea-estate labourers, and, when unripe or decomposed, is liable to cause enteritis.

*Kotchhu and Melostoma*.—Vegetation such as 'Kotchhu' and 'Melostoma' are, from our experience, not reliable as shade factors.

*For tanks and ponds.*

Bamboo matting about six feet wide can be anchored to the banks of the tanks and this effectually stops the breeding of *A. minimus*, although in most cases thorough cleaning of the banks is sufficient to limit or prevent breeding. The drawback to this latter method is that the process must be repeated every ten days to ensure success. Bamboo matting effectively meets the case and is cheap.

Tanks can be safely oiled provided the water is withdrawn by a pipe of which the inlet is at least 18 inches below the surface.

*For stagnant section drains in tea.*

Bamboo matting can also be used to cover narrow stagnant tea section drains, as this allows the drains to be cleaned out during the cold weather.

Occasionally we find in the tea districts, there exists a certain degree of prejudice against the establishment of Tarapat in hullahs as some planters consider that this is likely to lead to silting up of natural watercourses. Our experience does not support this belief. Where Tarapat has firmly re-established itself, we have never seen interference with the normal watercourse in any instance.

Drainage in tea gardens has always been a fetish in the past and much of the malaria in Assam can be attributed in many instances to a network of unnecessary drains, and the removal of the safe dense swamp vegetation from hullahs and swamps.

During the period between actual planting out and the time when the plants give dense and regular shade, temporary measures such as dusting of swamps with Paris green or oiling of channels are essential. It should be pointed out that oiling cannot be carried out in swamps as this would destroy the vegetation, but it can be readily applied to drains before shade is established. We would also stress the importance of protecting recently planted hedges and swamps with suitable fences from cattle trespass until the plants have grown sufficiently strong to withstand interference.

**FACTORS INFLUENCING THE BREEDING OF *ANOPHELES*.**

In our previous publications we have emphasized the various factors which influence the breeding of Anophelines. The character of the terrain has been carefully described, soil compositions have been accurately defined so as to show its influence on the water found in breeding areas, and particular attention has been drawn to the anti-larval action of silt in suspension. The distribution of rainfall, temperatures recorded during the year and relative and absolute humidities have been carefully studied. Records of sunshine and wind direction have also been carefully noted, as these are both closely correlated to cloud formation, the monsoon drift and precipitation of rain and dew.

Our infectivity survey has shown that *A. minimus* is practically the sole vector of malaria in the plains in Assam and the preference of this species for

human blood has been made evident. The habits of this species of breeding mainly in close proximity to human habitations have been noted and further observations during the year under review have corroborated these findings. Maps from scale survey plans have been prepared showing the courses of waterways both natural and artificial in all areas within the half-mile radius around each set of coolie lines. These maps show the summer and winter breeding resorts of *A. minimus*, and form the basis of the anti-larval work which was started in March 1931, and continued until the middle of November 1931. Anti-larval work was not carried out between the middle of November 1931 and 15th March, 1932. During this interval, the night temperatures fell below 60°F. During this period also, the summer resorts are dry and *A. minimus* larvæ are only found in permanent watercourses. In these, larval breeding continues uninterruptedly during the cold weather months, but as has already been pointed out, the larvæ are wintering, and remain in the larval stage for a very long period, at least not less than two months and most probably, in many instances, for a considerably longer period.

### ASTHENOBIOSIS.

The reasons for arrest of development in Anopheline larvæ present many interesting problems. The case of *A. umbrosus* has been particularly noted in this paper. Larval wintering in Assam begins roughly when the night temperatures have fallen consistently below 60°F., and continues until the night temperatures again rise above this level. Adults mainly hibernate during this period and it is only in warm cow-sheds or in heated human habitations that adults can be induced to feed.

The most obvious reasons for wintering are (i) a slowing up of the metabolic processes in the larvæ due to deficiency in the food supply, and (ii) a general decline in activity of larval life due to the lowering of the temperature.

In support of the theory that temperature and food supplies largely govern the process of larval maturation, we find that many larvæ, collected during the coldest period of the year, differ considerably from larvæ of the same species, collected during the warm monsoon period. This is particularly noticeable in the formation of the clypeal hairs which display, in many instances, fraying, branching or reduplication in species which normally have simple hairs. It would appear to us that this may be due to disease, analogous to the condition found in human hair and nails, when the individual is suffering from food deficiency, especially the absence of the A and D vitamins.

To us it seems evident that all life, vegetable or animal, is largely governed by the conditions enunciated in Van't Hoft's law :—

This law states that for each 18°F. rise in temperature, the speed of a chemical reaction is doubled and for a fall in temperature, the reaction is similarly slowed up. The correlation of maximum larval activity to maximum growth in the tea bush in Assam is very close and bears out the universal application of this law.



### COLD WEATHER CONTROL.

With relation to larval breeding in the cold weather, in the permanent water-courses, the question of anti-larval control during these months is closely associated. Is it wise to carry on anti-larval work throughout the year or is it sufficient to stop anti-larval operations when the adults of the malaria-carrying species become extremely scarce and larvæ are wintering? The question is of considerable importance in view of the extra expense incurred in carrying on anti-larval measures for twelve months instead of for eight months only.

On first consideration, it would seem a very sensible procedure to attack larval breeding areas in the cold weather period as these areas are limited in number and concentrated in a few main streams and collections of water. Control methods, in view of the long wintering of larvæ, could be applied at long intervals, say, once monthly during the months of December to February inclusive in Assam. Would the extra expenditure be justified and would treatment of breeding areas within the usual half-mile circle be effective? The answer to this question is dependent on the range of flight of adult mosquitoes during the periods of the year when mosquitoes are migrating to their winter resorts or returning to their summer breeding places.

The movements of Anophelines at various seasons of the year have been closely studied by us.

The possible range of flight of certain species of Anophelines is clearly shown in the dispersion of *A. gigas* and *A. maculatus* in Assam. *A. gigas* is a usual visitor in the cold weather months when there is need of fresh breeding areas owing to the drying up of the hill streams, although it is a hill mosquito which breeds at ranges of about 3,000 feet above sea-level and over. *A. maculatus* is also a casual visitor during the pre-monsoon months and is seldom found in the plains of Assam after May. The range of flight of these species means that both must travel many miles during the cold weather and pre-monsoon periods in search of suitable breeding areas. The distance from Jorhat to the nearest hills, where *A. gigas* breeds, is nineteen miles.

Again, in Northern Bengal we find hyperendemic malarious tea estates where the winter breeding places of the carrier species (*A. minimus*) are in some instances over eight miles distant from their normal summer resorts around the coolie line sites. This clearly shows the futility of controlling winter breeding places within a limited radius. The importance of treating summer resorts and the negligible results of treating winter resorts have already been emphasized in a previous paper (Ramsay, 1930).

### IMPORTANCE OF CONTROL STATIONS IN ANTI-LARVAL WORK.

From the foregoing it will be evident that 'control stations' are an important adjunct to anti-malarial control and these have been used in this district in conjunction with anti-larval measures. In some instances it has been found necessary to extend the area of anti-larval control beyond the usual half-mile circle in certain direction. The guiding factors are the findings in the

'control stations' and the wind direction. It is often possible to minimize extensions in other parts of the circle to less than the complete half mile, if healthy rice fields are present, or if suitable breeding places for the carrier species are not available. In the end, the area under control generally does not require more than the land surface contained in a circle of a half-mile radius, as economies on one side offset expenditure on another. In the Jorhat District larval counts, adult catches, and spleen and parasite rates are collected in the peripheral uncontrolled area within the triangle—Jorhat-Mariani-Titabar, and these afford excellent data for checking the results of the central controlled area. Controls must be established in connection with all anti-malarial schemes as otherwise results may be falsified by variations in climatic conditions, movements of 'non-salted' coolies and other conditions which contribute to the production of epidemic years.

To recapitulate, the knowledge essential for a successful anti-larval campaign comprises these factors :—

- (1) The possession of all data relating to the climatic conditions of the district, rainfall, temperature (maximum and minimum), relative and absolute humidity, wind direction, sunshine, physical geography and soil composition.
- (2) Spleen and parasite rates, malaria sick rates and sick rates from other causes, death rates due to malaria and death rates from other causes, also racial groupings.
- (3) Control stations *outside the area* where anti-larval measures are being attempted and control stations within the controlled area.
- (4) Thorough mapping of all areas, with *summer and winter* breeding resorts clearly defined.

### TEST OF RESULTS OF ANTI-LARVAL MEASURES.

The best indications in regard to success of anti-larval work are to be found in the reduction of spleen and parasite rates, reduction in total sickness, reduction in malarial sickness and reduction in both malarial and general mortality.

We realize that a period of at least five years should elapse before a true estimate of the value of a public health measure can be properly appraised and appreciated. We are therefore delaying publication of the excellent results which have so far accrued, although anti-larval measures would appear to be mainly responsible.

### SUMMARY AND CONCLUSIONS.

- (1) Dissection records continue to show that *A. minimus* is practically the only vector of malaria in Assam.

- (2) The bionomics of *A. umbrosus* and *A. ramsayi* have been carefully studied, but neither of these species appears to be of any sanitary importance in the malarial problem of the Sibsagar district.
- (3) The factors underlying dense shade as an anti-larval measure have been expressed. The covering of streams and other narrow water channels with dense shade eliminates the growth of chlorophyll containing vegetation which provides an anchorage for larvæ equipped with tail hooks.
- (4) Treatment of winter breeding resorts is not advocated owing to the fact that malaria is not being transmitted during the period of the year when the minimum temperatures are consistently below 60°F. Further, the length of flight of adult *Anopheles* from their winter breeding places to their summer resorts shows that the treatment of winter breeding resorts is of no practical value as an anti-malarial measure, in reducing the density of the adult mosquito population during the ensuing transmission season.
- (5) The anti-malaria measures which have been carried out have been described.

### ACKNOWLEDGMENTS.

We have to thank our Indian establishment, especially our Laboratory Assistant Dr. L. R. Dey and Malaria Surveyors Sachinandan Bhattacharjee and Mohammed Abdul Khaliq, for the great assistance they have rendered to us during this survey.

We have also to thank the Scientific Staff of the Tocklai Experimental Station, Jorhat, for much help and advice during our researches.

We also wish to record our appreciation of the help and co-operation we have received from the Managers of the various tea estates in the Cinnamara Medical Practice.

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## RICE CULTIVATION IN SPAIN, WITH SPECIAL REFERENCE TO THE CONDITIONS IN THE DELTA OF THE RIVER EBRO.\*

BY

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### *Introduction.*

THROUGH the kindness and financial assistance of the League of Nations, I was given an opportunity of visiting some of the rice-growing areas in Spain during the summer of 1931.

The duration of my tour was necessarily short, and it was impossible in the time at my disposal to become *au fait* with the many aspects of the malaria problems which occur in connection with rice cultivation in that country. Thanks to the valuable assistance given me by Dr. Sadi de Buen, Dr. Gil Collado, Dr. Torredemè, Dr. Peréperez and Dr. Serra, very much valuable information on the local malarial conditions was placed at my disposal. Dr. Pampana of the League of Nations accompanied me on my tour, and I am very grateful to him for all the help he gave me.

It is with some diffidence that I presume to give my impressions of the problems seen, but I do so in the hope that some of my suggestions may be helpful to other workers.

The main areas of rice cultivation visited were those at San Fulgencio (Alicante), the Gandia-Valencia area, the Prat el Llobregat near Barcelona and the Ebro Delta.

### RICE CULTIVATION IN SPAIN.

The problems of malaria in connection with rice cultivation in Spain, show a considerable difference from most of the problems with which I am familiar

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in connection with this form of agriculture in India. Aggregation of labour seems to play a very important part in epidemics of malaria in Spain, and only one species of Anopheline,\* *A. maculipennis*, is said to be implicated in connection with rice cultivation.

This form of agriculture in Spain is almost entirely confined to the alluvial tracts of deltaic areas, and the malarial problems presented in the different areas visited were very interesting. In some areas of rice cultivation malaria is an important problem, as in San Fulgencio, the 'Prat' el Llobregat, and the Ebro Delta. In other areas, however, the incidence of this disease is said to be negligible, for example in the Gandia-Valencia region.

During my tour in Spain the localities mentioned above were visited. Unfortunately on account of the dryness of the year, the areas at San Fulgencio and around Valencia were not being cultivated, so it was impossible to make any extensive personal observations in these places. It was possible, however, to make a closer study of the conditions in the Ebro Delta and in the Prat el Llobregat.

Most of the factors influencing the spread of malaria in any region consist of (i) a susceptible human population, (ii) a susceptible mosquito population with inclination and opportunities to bite man, (iii) the presence of human gametocyte carriers, and (iv) climatic or other conditions favourable for the development of the malarial parasite in the insect host and its transmission to man. Taking these factors into consideration, it is interesting to study their apparent relationship to the malarial conditions in rice-growing areas.

It seems possible to divide the history of rice cultivation in the regions visited in Spain into three very definite phases, each of which presents a different aspect :—

*Phase I.*—The period during which uncultivated and swampy localities are being converted into areas suitable for rice cultivation, i.e., levelling, filling, drainage, installation of irrigation, etc.

*Phase II.*—The period during which cultivation is commenced and the people are living under more or less primitive conditions.

*Phase III.*—The period when rice cultivation is well-established and the people are reaping in full the reward of their labours.

In addition to the three phases mentioned above, there is possibly another stage—*Phase IV*, during which there predominates a species or variety of Anopheline which has little or no inclination to bite man.

Most of my observations were made in areas where Phase II appeared to be merging into Phase III. The deductions concerning the other phases were chiefly made from the information placed at my disposal by various workers. I was unable to study a locality similar to Massarosa in Italy, where Phase IV appears to be acting as the cause of a low malarial incidence. It is possible that this stage may be present in the Gandia-Valencia area.

## RICE CULTIVATION (PHASE I).

If rice cultivation in any new area in Spain be started on a large scale, it appears to be invariably associated with a severe outbreak of malaria. This outbreak affects both the immigrant labour population and the local inhabitants. From the data available the following factors seem to have played, in the past, important rôles in the causation of these epidemics :—

(A) Aggregation of immigrant labour and the conditions associated with it.

(B) The opening-up of swamps and waste land, the introduction of more water, and the resultant disturbance of the established balance of the original indigenous flora and fauna of the area.

(C) The effects of the changes in the number and distribution of population, both human and animal, upon the distribution of Anophelines.

(A) *Aggregation of labour.*

This is a very important factor in the production of local epidemics of malaria in tropical countries. A very similar condition appears to occur in Spain, in connection with planting and harvesting operations and the initiation of large agricultural and industrial schemes. Many of the most malarious places visited seemed to be connected with this condition. In some other towns and villages, a greater or lesser proportion of the malarial incidence was attributed to the return of infected labour from such operations. This has caused not only a fictitious increase in the figures of locally acquired disease, but also a true increase in these, due to the rise in local infections as a result of the introduction of many gametocyte carriers with increased Anopheline infectivity.

Under conditions of aggregation of labour, the outbreak of malaria may be started in two different ways :—

(i) A group of workmen coming from an area where malaria is slight or absent (*i.e.*, non-immunes), are introduced into a locality where the disease is rife, or

(ii) Groups of workmen, from many different places where malaria is prevalent, are introduced into a locality where the incidence of the disease is low, but where the conditions engendered by the work in progress are highly favourable for the spread of the disease (*i.e.*, a great increase in the numbers of carrier Anophelines and of human gametocyte carriers).

In the former instance the brunt of the epidemic will probably fall primarily on the immigrant population, and later affect the local inhabitants. In the latter instance the outbreak will affect both populations eventually, but it is probable that the local or non-immune population will suffer most in the initial stages.

Sometimes this immigrant population is drawn largely from areas where economic stress is present, because the contractors can obtain cheaper labour in this way. Such *cheapness of labour*, however, often means that the workmen have to live under very poor conditions of diet, sanitation and housing, because they are unable to afford anything better on the wage received. Apart from

the question of wages, the arrangements for the accommodation and feeding of labour are often, of necessity, primitive at the commencement of any large undertaking in a remote locality. These adverse conditions usually mean a lowered resistance of the worker, and poor facilities for the treatment of any infection he may acquire.

*Shortage of accommodation*, more especially in the initial stages, leads to overcrowding. This may exercise its deleterious effects in a variety of ways :—

(i) It facilitates the spread of infections, because one infected mosquito in a room or barrack will be liable to infect more than one person.

(ii) Conversely a larger number of mosquitoes are liable to acquire the infection from one human gametocyte carrier than if there be fewer persons present.

(iii) The chances of a mosquito acquiring an infection rise with the number of feeds it gets on an infected person. These chances rise with overcrowding.

(iv) The severity of an infection in man appears to increase with the number of bites he receives from an infected mosquito. Multiple bites are much more likely to occur under the conditions mentioned.

(v) The presence of multiple human gametocyte carriers from different areas and of multiple infected insects, makes the chance of infection with a number of strains\* or species of parasite more probable.

(vi) The close association between the insect and human carriers under such conditions, is ideal for rapid and repeated transmission of the parasite. Such transmission may possibly cause an increase in the virulence of the parasite, although the evidence on this point is not conclusive (Sinton, 1931).

All these factors play important parts in the causation of malarial outbreaks under conditions of labour aggregation. The introduction of a great variety of 'foreign' strains of malarial parasite has probably a very important bearing on the severity of the attacks of the disease, and this has apparently a very close relationship with the number of gametocytes produced by any attack (Sinton, 1926).

When the constructive operations are started and are in progress, the *habitations* are usually collected into large groups. The location of these groups is determined by the position of available and suitable ground, and its relation to the work in progress. These areas are often chosen near the sites occupied by the local inhabitants. This facilitates the passage of infection between immune and non-immune populations. Many of the factors associated with overcrowding also come into action.

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\*The probable importance of the strain of parasite in relation to the severity of the malarial attack, and to the rate of clinical or permanent cure of the disease, has been discussed elsewhere (Sinton, 1931). The great influence of this factor in connection with severity of the attack in super-infections by different strains of parasite, has been confirmed by recent researches on monkey malaria in these laboratories (Mulligan and Sinton, 1933).

If suitable malaria-carrying insects be present, the operations needed in preparing an area for rice cultivation are usually those suitable for a great *local increase of such insects* (*vide infra*).

The main factors responsible for those outbreaks of malaria, which are associated with aggregation of labour in malarious regions, may be summarised as follows :—

(1) A large non-immune or partially immune population introduced into a highly malarious area, or a highly malarious population introduced into an area of low or high malarial endemicity.

(2) A great local increase in the numbers of either insect or human carriers of malaria, or both.

(3) Poor economic conditions causing a lowered resistance of the population to the disease.

(4) An increased severity of the clinical symptoms of malaria, with a consequent increase in the numbers of gametocyte carriers and of the numbers of gametocytes in the blood of each infected person. This may be due to—

(i) Lowered resistance of the population.

(ii) Primary attacks of malaria in a non-immune population.

(iii) Introduction of many different strains of parasite, or enhanced virulence of the local strains.

(iv) Greater chances of multiple infections with different strains and species of parasite, as well as an increased dosage of sporozoites due to multiple bites.

(5) Overcrowded accommodation in houses and close grouping of dwellings, facilitating the passage of infection from man to man.

(6) Absence of facilities for good and prompt treatment, and for the nursing of sick persons.

(B) *Opening-up of swamps, etc., and introduction of irrigation.*

Apart from the great increase in the number of breeding-places for mosquitoes caused by such operations, several other factors may come into play.

These factors are chiefly connected with the disturbance of the natural balance, which has been established from time immemorial between the various types of flora and fauna in the area.

During the primary stages of preparation for rice cultivation, the drainage, filling, levelling, clearing, etc., causes marked changes in the local vegetation, not only the terrestrial but also the aquatic forms. The changes produced, such as exposure of water to sunlight, etc., may result in an enhancement of the breeding conditions of various mosquitoes, and thus an increase in their numbers. The change from the permanent aquatic conditions of swamps, etc., to the intermittent ones of rice cultivation, must necessarily have an influence on the type and abundance of the various aquatic flora and fauna.

The filling-in of swampy areas may result in the destruction of large numbers of the natural enemies of mosquitoes, while the changed conditions of



newly-formed collections of water may not be suitable for the survival and multiplication\* of these enemies. This will affect the natural balance established between the number of mosquitoes and their enemies, with a resultant benefit to the former.

The destruction of terrestrial vegetation removes many of the places in which adult mosquitoes naturally shelter. Suitable shelter, more especially in close relationship to a good food supply, is a very important factor in the life history of *A. maculipennis*. The result is that these mosquitoes tend to crowd into the human habitations, and the latter on account of their primitive character, are usually eminently suited to shelter the insects.

*(C) Changes in population in relation to Anopheline distribution.*

In the early stages of such work few or no domestic animals are present in proportion to the human population. The flocks and herds, which under ordinary conditions roamed over the area, are taken further afield as their grazing grounds are destroyed or limited by the work in progress. This means that there are few or no domestic animals to divert the attentions of the mosquitoes, with a resultant intensity of their attacks on man.

*Preventive measures in Phase I of rice cultivation.*

During this first phase of rice cultivation, the operations for which the workmen are collected are of a temporary nature, while the groups of workmen are in many instances widely scattered and frequently moved from place to place. Under such conditions it does not seem that the prevention of malaria by anti-larval measures alone, is a feasible financial proposition. Some system of controlled medication, both curative and prophylactic of symptoms, appears in our present state of knowledge to be the most suitable, as the basis of prevention under these conditions.

If such operations be financed by a wealthy company, the erection of mosquito-proofed houses might be considered. These might be built on such a plan, and in such localities, as would make them suitable for later permanent occupation by the new settlers. These buildings would, however, need to be made prior to any large influx of people, so that many conditions associated with and following upon aggregation of labour, would be less likely to occur.†

RICE CULTIVATION (PHASE II).

When the major work of preparation of an area for rice cultivation is finished, another phase is entered upon.

\* The workmen make every effort to supplement their scanty diet. Netting and other methods of fishing are largely used, without any regard to the size of the fish captured. This also helps to cause a decrease in the numbers of larvicidal fish.

† At Macarese in Italy, 'bonification' along these lines is proceeding.

In this second phase the bulk of the imported labour departs and labour immigration is only present in any large amount at special seasons, to help with agricultural operations such as the planting and harvesting of crops. This seasonal re-introduction of a floating population is necessary, because the number of local inhabitants is not large enough, at this early period of colonisation, to supply the demand for workers.

The original workmen will have left their legacy of disease behind them and the infected or susceptible new comers will tend to prevent this from dying down rapidly.

The social and economic conditions of the local inhabitants at this period will also be conducive to a continuation of a high incidence of malaria. The outlay incurred by them in acquiring their holdings, and the various initial expenses in connection with rice cultivation, will leave them in a low financial state, in many instances. Such conditions will continue until the pecuniary benefits of several harvests have been felt.

These people will probably occupy at first the old habitations used by the original workmen, until they can build more suitable shelter for themselves. This will make them liable to infection and reinfection from the local mosquitoes. When they build their own houses, these will at first be small and conditions of overcrowding common.

The adverse conditions of poor food and overwork will continue. Money to buy cattle, and house them properly, will be scarce, so zoophilism will have little effect. The people will be able to afford little medicine or medical advice, even if this was readily available in a newly-settled area. For the latter reasons, efficient treatment will be rare, malarial morbidity high, and gametocyte carriers common.

During this phase, the adverse conditions will resemble those seen in the first phase but to a less degree, while the more favourable circumstances of the third phase will not have commenced to operate to any very appreciable extent.

#### *Preventive measures in Phase II of rice cultivation.*

The main scheme of malarial control in this phase would seem to be very similar to that in the first phase, *i.e.*, proper treatment of malarial cases. This should be supplemented, where possible, by a financial subsidy to enable the settlers rapidly to obtain proper housing, sufficient food, a supply of cattle, *etc.*, *i.e.*, a 'bonification', such as is being aimed at in the work at Macarese near Rome.

#### RICE CULTIVATION (PHASE III).

In old and well-established areas of rice cultivation malaria is said to be a negligible problem. This may be called the third phase.

In the years which have elapsed since rice cultivation was started, a poverty-stricken population has become a prosperous one. With this condition of 'bonification', most of the adverse influences which acted as causal factors

of high malarial incidence, have disappeared. The people are well-housed in habitations less attractive for the shelter of mosquitoes. Overcrowding has diminished. Screening has been attempted in some dwellings.\* Over-work and the scarcity of good food have largely disappeared. Domestic animals are plentiful and are housed under conditions which attract mosquitoes for shelter.† The sick can afford to avail themselves of prompt and effective treatment. With the natural increase of the population, the demand for immigrant labour diminishes with a resultant decrease in the introduction of infection.

No opportunity was available for studying the village conditions in old rice areas where malaria has disappeared, as in the Gandia-Valencia district. The area of rice cultivation at La Cava in the Ebro Delta was visited. Here, probably largely as the result of the treatment campaign in force, the conditions seemed to be progressing rapidly towards the third phase. Valuable information was collected in this locality, which would appear to indicate the manner in which this phase develops.

At La Cava most of the farmers live in scattered and isolated dwellings in the midst of a sea of rice. These dwellings are mostly well built and in some cases of two storeys. Screening has been attempted in some instances to ameliorate the mosquito nuisance. These habitations are closely surrounded by the housing for domestic animals, which usually affords ideal conditions for the shelter of Anophelines. A few years ago malaria was very prevalent here but, probably as a result of the treatment campaign, it has diminished very considerably. The anophelism is so intense as to be almost unbelievable.

Enquiries made locally elicited some very interesting information, which would appear to indicate the method of spread of malarial infection in this area.

In any of these isolated habitations, no malaria might occur for a year or more, in spite of the intense anophelism. On the other hand, if a human malaria carrier be introduced, the disease usually spreads through the entire household. The introduced carrier might be either an imported labourer, or a member of the family who had picked up the disease in the local village or some other place.

This infection appeared to remain localised and not to spread to neighbouring dwellings several hundred yards away. The following data about the mosquito population would appear to account for this localisation of infection :—

(a) Numerous suitable breeding places were present in the rice fields in the immediate vicinity of the dwellings.‡

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\* With the large number of suitable and easily accessible domestic animals, even a moderate degree of screening would seem to be sufficient to deter *A. maculipennis* in Spain from entering these human habitations in any large numbers.

† In areas of rice cultivation, the animals' houses are grouped around human habitations, as this is often the only area not liable to periodical flooding during the agricultural operations.

‡ Dr. Gil Collado informed me that Anopheline larvæ in this area were more plentiful near dwellings than further afield, an observation which supports the view that the insects do not wander far under conditions apparently so satisfactory for them.

(b) The food supply is plentiful in the form of domestic animals.

(c) Suitable shelter is abundant and easily accessible, in the form of the dark and warm animal houses.

These conditions appear to form an ideal environment for the life cycle of the mosquito, *A. maculipennis*, and there would seem to be little need or cause for the insects to wander further afield.\* Under these circumstances there seems little chance of the infection spreading to other distant houses. Even if an infected Anopheline should be carried or wander to another area, the influence of zoophilism, as well as the deterrent effect of screening, would greatly limit its chances of biting another human being.

In this area, therefore, while the insect factor is eminently suited for the carriage of the disease, mosquitoes would appear to be merely a nuisance and not an actual danger *until* a human carrier is introduced. With the proper treatment of malarial cases and steps to prevent the introduction of gametocyte carriers,† there would be a tendency for the disease to die out in such localities.

Under normal circumstances the change from Phase II to Phase III would only take place very gradually over a period of many years, during which the factors favouring the incidence of the disease diminished. In La Cava the arrival of this third stage is being accelerated by the treatment campaign‡

In some of the older rice-field areas cultivation is gradually spreading. Here no serious outbreaks of malaria have been reported similar to those which marked the original undertaking in these localities. This is what might be expected, because the extension is largely carried out by local labour. In this way the dangers attendant upon aggregation of imported labour have been diminished, while the condition of the local people would also be less suitable for the occurrence of an epidemic. This absence of serious outbreaks may, however, be partly attributable to the greater precautions (medical treatment and prophylaxis), which have been taken in recent years when any such outbreak was feared.

\* From the information available about *A. maculipennis*, the range of flight of this insect in any locality under normal conditions, would appear to be the distance to the nearest suitable food supply and to suitable breeding grounds.

† Routine examination of immigrant labour and treatment of infected cases is carried out in the Prat el Llobregat rice-area.

‡ This position with regard to malarial incidence appears to be very similar to that which forms the basis of the work in the new Italian colony at Macarese. At the latter place the new farm houses are collected in isolated centres, and screened accommodation is provided both for the colonists and the immigrant labour. Treatment, both curative and prophylactic, is compulsory for all inhabitants of the area. The antilarval measures in force around the central village would tend to diminish the chances of acquiring an infection, when the inhabitants of the rural areas visit this place. Apart from these purely direct measures, the new colonists receive a subsidy to tide them over the first lean years. This work might be called an attempt to obtain rapidly a condition similar to Phase III of rice cultivation in Spain.

The conditions of rice cultivation in the Prat el Llobregat have a closer resemblance to Phase II than to Phase III. Whereas at La Cava the cultivation is done by peasant farmers, in the 'Prat' several large farmers have been licensed to undertake rice cultivation, and the work is mostly carried out by imported labour. This means that there is a continued influx of infected labour as in Phase II. To overcome this danger, all labour working in rice-fields must be medically examined. The result of the examination is entered on a card, which the labourer must produce on demand. If he be found to have malaria, he is given the choice between a full course of treatment, and being returned to his home at the farmer's expense. All rice-field labourers must take prophylactic quinine. If one should contract malaria he receives free treatment and is paid while out of work.

#### RICE CULTIVATION (PHASE IV).

It seems probable that a fourth stage may occur in the history of rice cultivation. In this stage, while numerous Anophelins are present, malaria is absent ('Anophelism without malaria').

This condition has been explained as due either to (i) the development of a larger and more robust type of insect, the intestinal mucosa of which offers a greater resistance to the invasion of the malaria parasite, or (ii) the comparatively rapid\* appearance of a new zoophilic race of *A. maculipennis*, which seldom or never attacks man, if the blood of domestic animals be available. This race is supposed to have displaced an androphilic one.

A typical example of this phase would appear to occur at Massarosa in Tuscany. As the race of *A. maculipennis* from this area has proved normally susceptible to malarial infection, and is used extensively for experimental malarial transmission in connection with the treatment of general paralysis of the insane, no support is given to the first explanation.

As my only experience of such an area was the visit to Massarosa, so kindly arranged for me by Prof. Missiroli and Dr. Hackett, it is with considerable hesitancy that I put forward some suggestions in connection with this phenomenon.

While very much evidence has been produced to support the second hypothesis, this does not appear to me to have covered all the possibilities of the situation. It is quite possible that the changed conditions of the breeding places, which eventually develop in old-established areas of rice cultivation, and the greater facilities for feeding on domestic animals which have arisen, may make the region more suitable for a biologically different type of mosquito. It may also be that, in a comparatively short\* period, there has been evolved a zoophilic race of *A. maculipennis*, or that such a race has replaced or displaced an androphilic one.

On the other hand, it is well known in malariology that changes in the nature of breeding places may result in the disappearance of one species of

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\* 'Rapid' or 'short' from the point of view of evolution.

Anopheline with a marked increase of another species. It, therefore, seems to me quite possible that the two types of insect reported may really be two different *species*, rather than two *races* of the same species with rapidly evolved bionomical differences.

Already the species, *A. maculipennis*, is being divided on morphological differences into several varieties by European entomologists. Even within the last few years, *A. elutus* has been separated from the *maculipennis* group as a distinct species, both morphologically and bionomically. The *rossi-ludlowi* group of Oriental Anophelines has been found, on recently discovered morphological differences, to be composed of many more species than those previously recognised. These species have also been found to vary considerably both in their bionomics and their potentialities for the carriage of malaria.

The evidence that such a zoophilic change occurs in the habits of the local Anophelines in some areas during a comparatively short time, cannot be neglected. It seems to me, however, that there is as yet insufficient evidence to prove that this change is not due to the replacement of one distinct species by another, rather than to a rapid change in the habits of individuals of the same species. It is easily conceivable that such a replacement of species might follow upon the changes in environment, which have occurred when a deserted and swampy area is converted into a populous and cultivated rice country.

If the phenomenon be due to a replacement of species or races, it might be possible to discover the biological or other conditions which govern this change, and thus hasten their production as a means of malarial control. On the other hand, if the change be due to the evolution of a new race, the process would be slower and more difficult to govern or accelerate. Whatever the reasons of this change may be, its completion seems too slow and its causes too obscure, to make it form a practical method of malarial control in our present state of knowledge. Further research may, however, elucidate the mystery and devise some practical measure of malarial control from the results obtained.

While this replacement of species or races of Anopheline, may be the only cause of the disappearance of malaria in areas like Massarosa in Italy, there are possibly other contributory factors. The conditions in these old areas of rice cultivation may be merely an extension of the circumstances described in Phase III. The extensive quinine campaign which has been carried out in Italy during the last 30 years may have helped the fall in malarial incidence, by causing a decrease in the number of malarial carriers in the Massarosa area. A decrease in the introduction of gametocyte carriers may also have helped in the matter, for apparently in some areas where such carriers were introduced after the War, malaria was again reported. I have not, however, sufficient information on these points to discuss them more fully, and merely put them forward as suggestions.

Whether Phase IV exists in any of the areas of rice cultivation in Spain I am unable to say, but the conditions reported in the Gandia-Valencia area suggest that it may be present there.

*Conclusions.*

Three different phases of malarial incidence, and possibly a fourth, appear to occur in the history of rice cultivation in Spain.

The malarial incidence in the first phase seems to be caused mainly by aggregation of labour and its associated conditions.

The second phase appears to be the aftermath of the first. The malarial incidence is dependent upon factors connected with poor economic conditions and the gametocyte-carrier problem.

The low incidence recorded in the third stage follows as the result of the disappearance of many of the factors associated with poor economic conditions, and a diminution in the intensity of the gametocyte-carrier problem.

If a fourth stage be present in the older areas of rice cultivation in Spain, it may be associated with the gradual predominance of a species or race of *Anopheles* with marked zoophilic habits.

The first step to be taken in controlling the malarial incidence in areas of rice cultivation in Spain would seem to be measures for mass treatment of the disease. These should always be reinforced by measures for the elimination or exclusion of gametocyte carriers.

Where possible, the dangers associated with aggregation of labour should be counteracted, and all practicable steps taken to hasten the conditions of 'bonification' found in the third stage of rice cultivation.

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*Idem* (1931) .. .. *Ibid.*, **18**, 3, pp. 845-853.

ADDITIONAL RECORDS OF THE DISTRIBUTION OF  
ANOPHELINE MOSQUITOES IN INDIA (FROM  
JANUARY 1, 1931 TO APRIL 15, 1933).

BY

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[April 28, 1933.]

SINCE the publication by Major G. Covell, I.M.S., of two papers (Covell 1927 and 1931) on the distribution of Anopheline mosquitoes in India and Ceylon, more than 56,000 mosquitoes and mosquito larvæ have been identified by the Malaria Survey of India (from 1st January 1931 to 15th April 1933). The large majority of these were Anophelines, and about 25,000 of the specimens were received at Kasauli, chiefly from workers in various parts of India. The remainder were nearly all collected in the Karnal District, Punjab, and identified at the Ross Field Experimental Station for Malaria, Karnal. Many of the specimens were from places not recorded in Covell's two papers referred to above. In the present paper only *new* records are given, and these are confined to specimens which have been entered in the registers and collecting books of the Malaria Survey of India, in the course of routine identifications during the period mentioned above.

In cases where previously a species was recorded from a District only, and not from a particular place, or the record was considered doubtful, further records have been given, where such were available.

The numbers given after each record are the reference numbers in the Central Malaria Bureau registers. When specimens are retained for the Kasauli collections they are labelled with these numbers.

The arrangement of Divisions and Districts, as used by Covell, has been followed, except that Sirohi State, including Mount Abu, has been added, under Rajputana (West) Division.

The following recent changes in nomenclature have been adopted :—  
**A. annularis** v. d. Wulp. (*A. fuliginosus* Giles), **A. fluviatilis** James (*A. listonii* Liston), **A. splendidus** Koidzumi (*A. maculipalpis* var. *indiensis* Theo.), **A. sundanicus** Rodenwalt (*A. ludlowii* Theo.).



**aconitus.****ASSAM.****Sylhet Dist., Sylhet, 194-32.****BENGAL.****Calcutta, Garden Reach, 53-32.****BIHAR.****Darbhanga Dist., Madhubani, 274-32, 3-33.****HYDERABAD (SOUTH).****Atraf-i-Balda Dist., Hyderabad, 54-31.****MADRAS COAST (NORTH).****Vizagapatam Dist., Mattili, 175-32.****aitkenii.****ASSAM.****Naga Hills Dist., Kohima, 202-32.****MADRAS COAST (NORTH).****Vizagapatam Dist., Govindpalli, 175-32.****MALABAR.****Travancore State, Vandiperiyar, 256-31.****ORISSA.****Kalahandi State, Ambadola, 54-32.****aitkenii var. pinjaurensis.****PUNJAB (EAST & NORTH).****Patiala State, Pinjaur, 283-32.****annularis (fuliginosus).****ASSAM.****Lakhimpur Dist., Jeypore, 128-31.****BENGAL.****Birbhum Dist., Sriniketan, near Surul, 142-32, 143-32.**

**BIHAR.**

**Darbhanga Dist.,** Madhubani, **242-32, 249-32, 274-32, 3-33, 22-33;**  
**Shahabad Dist.,** Dehri-on-Sone, **213-32, 260-32.**

**BOMBAY DECCAN.**

**Sholapur Dist.,** Kurduwadi, **52-33.**

**BURMA (LOWER).**

**Hanthawaddy Dist.,** Mingaladon, **161-32.**

**CENTRAL INDIA (EAST).**

**Bundelkand Agency,** Nowgong, **242-31, 15-32.**

**CENTRAL PROVINCES (WEST).**

**Jubbulpore Dist.,** Jubbulpore, **29-31, 9-32.**

**CHOTA NAGPUR.**

**Manbhum Dist.,** Bhaga (Jherria Coal-fields), **47-31; Ranchi Dist.,**  
**Kamdera, 259-32.**

**HYDERABAD (SOUTH).**

**Atraf-i-Balda Dist.,** Hyderabad, **54-31.**

**MADRAS COAST (NORTH).**

**Vizagapatam Dist.,** Boipariguda, **175-32, Govindpalli, 175-32, Jeypore**  
**Town, 179-32, Mattili, 175-32, Pujariguda, 175-32.**

**NORTH WEST FRONTIER PROVINCE.**

**Chitral State,** Drosh, **234-31.**

**PUNJAB (EAST & NORTH).**

**Ambala Dist.,** Ambala, **5-31, 17-32, etc.; Karnal Dist.,** Barauta, **64-33,**  
**Budha Khera, 63-33, Dabri, 63-33, Dadupur, 68-33, Darar, 60-33, Gagsina,**  
**69-33, Ghogripur, 57-33, Indri, 56-33, Jhanjari, 66-33, Kambohpora, 61-33,**  
**Munak, 67-33, Rambha, 70-33, Saidpura, 55-33, Shahpur, 65-33, Taraori,**  
**62-33; Patiala State,** Badshahpura, **158-32, Narnaul, 158-32, Pinjaur, 65-31,**  
**283-32, Rajpura, 158-32, Sutariana, 158-32.**

**RAJPUTANA (EAST).**

**Jaipur State,** Sambhar Town, **95-32.**

**RAJPUTANA (WEST).**

**Sirohi State,** Mount Abu, **86-32, 90-32, 97-32.**

## UNITED PROVINCES (EAST).

**Gorakpur Dist., Gorakpur, 281-32, 8-33, 14-33, 24-33, 42-33.**

## UNITED PROVINCES (WEST).

**Aligarh Dist., Aligarh, 229-32; Jhansi Dist., Jhansi, 25-32, 38-32, 57-32, 84-32; Meerut Dist., Babugarh, 272-31.****barbirostris.**

## ASSAM.

**Lakhimpur Dist., Jeypore, 128-31, Margherita, 238-31, Namsang, 128-31; Sibsagar Dist., Deopani Tea Estate, 180-31; Sylhet Dist., Sylhet, 194-32.**

## BENGAL.

**Birbhum Dist., Sriniketan, near Surul, 143-32.**

## BIHAR.

**Darbhanga Dist., Madhubani, 242-32, 249-32, 274-32, 3-33, 22-33.**

## BOMBAY DECCAN.

**Sholapur Dist., Kavitgaon, 34-31, Kurduwadi, 7-31.**

## BURMA (LOWER).

**Hanthawaddy Dist., Mingaladon, 43-31.**

## PUNJAB (EAST &amp; NORTH).

**Karnal Dist., Barauta, 64-33, Buddha Khera, 63-33.****barbirostris var. ahomi.**

## ASSAM.

**Lakhimpur Dist., Margherita, 28-32.****culicifacies.**

## ASSAM.

**Lakhimpur Dist., Namsang, 128-31; Sibsagar Dist., Deopani Tea Estate, 180-31; Sylhet Dist., Sylhet, 43-33.**

## BIHAR.

**Darbhanga Dist., Madhubani, 249-32, 3-33, 22-33; Shahabad Dist., Deri-on-Sone, 248-32, 260-32.**

## BOMBAY DECCAN.

**East Khandesh Dist., Bhusaval, 14-32; Sholapur Dist., Bhamburdi, 34-31, Kavitgaon, 34-31, Khudus, 34-31, Malaoli, 34-31, Mandwe, 34-31.**

**CENTRAL PROVINCES (WEST).**

**Chhindwara Dist.,** Kukra Khapa, **153-31; Saugor Dist.,** Bina, **181-32.**

**CHOTA NAGPUR.**

**Manbhum Dist.,** Bhaga (Jherria Coal-fields), **47-31; Palamau Dist.,** Barwadih, **131-31, 198-32, 211-32, 241-32; Ranchi Dist.,** Kamdera, **259-32; Singhbhum Dist.,** Naomundi, **36-31.**

**HYDERABAD (SOUTH).**

**Atraf-i-Balda Dist.,** Hyderabad, **54-31.**

**MADRAS COAST (NORTH).**

**Vizagapatam Dist.,** Govindpalli, **175-32, Jeypore Town, 179-32, Pujariguda, 175-32.**

**PUNJAB (EAST & NORTH).**

**Ambala Dist.,** Barara, **9-33; Jhelum Dist.,** Jhelum, **182-31; Karnal Dist.,** Barauta, **64-33, Budha Khera, 63-33, Dabri, 63-33, Dadupur, 68-33, Darar, 60-33, Gagsina, 69-33, Ghogripur, 57-33, Indri, 56-33, Jhanjari, 66-33, Kamboh-pura, 61-33, Kunjpura, 58-33, Munak, 67-33, Rambha, 70-33, Saidpura, 55-33, Shahpur, 65-33, Taraori, 62-33; Patiala State, Badshahpura, 158-32, Mohindar Garh, 158-32, Narnaul, 158-32, Rajpura, 158-32, Sutariana, 158-32.**

**RAJPUTANA (EAST).**

**Ajmer-Merwara Province, Ajmer, 78-32, 96-32.**

**RAJPUTANA (WEST).**

**Sirohi State, Mount Abu, 86-32, 90-32, 97-32.**

**UNITED PROVINCES (EAST).**

**Cawnpore Dist., Cawnpore, 117-31.**

**UNITED PROVINCES (WEST).**

**Jhansi Dist., Jhansi, 325-31, 25-32.**

**dthali.**

**BALUCHISTAN.**

**Quetta-Pishin Dist., Chaman, 132-31.**

**fluviatilis (listonii).**

**BIHAR.**

**Darbhanga Dist., Madhubani, 3-33.**

**BOMBAY DECCAN.**

**Sholapur Dist., Mandwe, 34-31.**

**CENTRAL INDIA (EAST).**

**Bundelkand Agency, Nowgong, 354-31.**

**CHOTA NAGPUR.**

**Manbhum Dist., Bhaga (Jherria Coal-fields), 47-31; Palamau Dist., Barwadih, 241-32; Ranchi Dist., Kamdera, 259-32.**

**HYDERABAD (SOUTH).**

**Atraf-i-Balda Dist., Hyderabad, 54-31.**

**MADRAS COAST (NORTH).**

**Vizagapatam Dist., Govindpalli, 175-32, Mattili, 175-32, Pottangi, 175-32.**

**ORISSA.**

**Kalahandi State, Dabriguda, near Ampani, 67-31.**

**PUNJAB (EAST & NORTH).**

**Ambala Dist., Barara, 9-33, Jagadhri, 9-33; Karnal Dist., Barauta, 64-33, Ghogripur, 57-33, Jhanjari, 66-33, Kambohpora, 61-33, Karnal, 59-33, Said-pura, 55-33, Taraori, 62-33; Simla Dist., Solan, 298-31.**

**RAJPUTANA (WEST).**

**Sirohi State, Mount Abu, 97-32.**

**UNITED PROVINCES (EAST).**

**Gorakpur Dist., Gorakpur, 281-32, 8-33.**

**WAZIRISTAN.**

**Derajat Area, Wana, 245-31, 75-32, 138-32.**

***gigas*, including vars.**

**ASSAM.**

**Goalpara Dist., Kachugaon, 27-33; Lakhimpur Dist., Margherita, 13-32, 28-32.**

**MADRAS (SOUTH-EAST).**

**Nilgiris Dist., Wellington, 7-32.**

**NORTH WEST FRONTIER PROVINCE.**

**Swat Territory**, Malakand, **79-31.**

**PUNJAB (EAST & NORTH).**

**Patiala State**, Pinjaur, **368-31.**

**hyrcanus** var. **nigerrimus.**

**ASSAM.**

**Lakhimpur Dist.**, Jeypore, **128-31, 267-32**, Margherita, **238-31**, Tinsukia, **267-31**; **Sibsagar Dist.**, Deopani Tea Estate, **180-31**; **Kamrup Dist.**, Khanapara, **230-31.**

**BENGAL.**

**Birbhum Dist.**, Sriniketan, near Surul, **142-32.**

**BIHAR.**

**Darbhanga Dist.**, Madhubani, **249-32, 3-33, 22-33.**

**BURMA (LOWER).**

**Hanthawaddy Dist.**, Mingaladon, **43-31, 161-32.**

**CENTRAL PROVINCES (WEST).**

**Jubbulpore Dist.**, Jubbulpore, **29-31**; **Saugor Dist.**, Bina, **181-32.**

**CHOTA NAGPUR.**

**Ranchi Dist.**, Kamdera, **259-32.**

**HYDERABAD (SOUTH).**

**Atraf-i-Balda Dist.**, Hyderabad, **54-31.**

**MADRAS COAST (NORTH).**

**Vizagapatam Dist.**, Jeypore Town, **179-32**, Mattili, **175-32**, Pujariguda, **175-32.**

**ORISSA.**

**Kalahandi State**, Dabriguda, near Ampani, **67-31.**

**PUNJAB (EAST & NORTH).**

**Karnal Dist.**, Barauta, **64-33**, Indri, **56-33**, Rambha, **70-33**, Saidpura, **55-33**, Taraori, **62-33**; **Rawalpindi Dist.**, Rawalpindi, **171-32.**

**UNITED PROVINCES (WEST).**

**Aligarh Dist.**, Aligarh, **229-32**; **Saharanpur Dist.**, Roorkee, **288-31.**

**jamesii.****ASSAM.****Sylhet Dist., Sylhet, 194-32.****BENGAL.****Birbhum Dist., Sriniketan, near Surul, 143-32.****BOMBAY DECCAN.****Poona Dist., Poona, 257-31, 21-33; Sholapur Dist., Kurduwadi, 349-31.****BURMA (LOWER).****Hanthawaddy Dist., Mingaladon, 43-31.****CHOTA NAGPUR.****Ranchi Dist., Kamdera, 259-32.****GUJARAT.****Baroda State, Baroda, 243-32.****MADRAS COAST (NORTH).****Vizagapatam Dist., Ambadola, 273-32, Mattili, 175-32, Pujariguda, 175-32.****jeyporiensis.****ASSAM.****Sylhet Dist., Sylhet, 218-32.****BOMBAY DECCAN.****Dharwar Dist., Hubli, 41-32.****CHOTA NAGPUR.****Ranchi Dist., Kamdera, 259-32.****KONKAN.****Savantvadi State, 363-31, 10-32, 12-32, 32-32, 121-32, 31-33.****MADRAS COAST (NORTH).****Vizagapatam Dist., Boipariguda, 175-32, Govindpalli, 175-32, Mattili, 175-32, Pottangi, 175-32, Pujariguda, 175-32.****karwari.****ASSAM.****Sibsagar Dist., Deopani Tea Estate, 180-31; Sylhet Dist., Sylhet, 194-32.**

**kochi.**

**ASSAM.**

**Goalpara Dist.,** Kachugaon, **229-31**; **Kamrup Dist.,** Khanapara, **230-31**;  
**Lakhimpur Dist.,** Jeypore, **128-31**, Namsang, **206-31**.

**leucosphyrus.**

**MALABAR.**

**Travancore State,** Vandiperiyar, **256-31**.

**lindesaii.**

**ASSAM.**

**Naga Hills Dist.,** Kohima, **202-32**.

**PUNJAB (EAST & NORTH).**

**Patiala State,** Pinjaur, **283-32**.

**maculatus, and**  
**maculatus var. willmori.**

**ASSAM.**

**Goalpara Dist.,** Kachugaon, **154-31**; **Lakhimpur Dist.,** Namsang, **128-31**;  
**Sylhet Dist.,** Sylhet, **253-32**.

**BENGAL.**

**Jalpaiguri Dist.,** Chuapara, **282-32**.

**BIHAR.**

**Darbhangu Dist.,** Madhubani, **249-32**.

**NORTH WEST FRONTIER PROVINCE.**

**Peshawar Dist.,** Dargai, **101-32**.

**ORISSA.**

**Kalahandi State,** Dabriguda, near Ampani, **67-31**.

**PUNJAB (EAST & NORTH).**

**Ambala Dist.,** Ambala, **31-32**; **Gurdaspur Dist.,** Dunera, **152-32**; **Sialkot Dist.,** Sialkot, **46-33**.

**WAZIRISTAN.**

**Derajat Area,** Wana, **68-32**.



**minimus.****ASSAM.**

**Sibsagar Dist.**, Cinnamara, **196-32, 201-32, 234-32**, Deopani Tea Estate, **180-31**; **Sylhet Dist.**, Sylhet, **253-32**.

**KONKAN.**

**Savantvadi State**, **10-32**.

**MADRAS COAST (NORTH).**

**Vizagapatam Dist.**, Chatikona, **38-31**, Pottangi, **175-32**.

**UNITED PROVINCES (EAST).**

**Gorakpur Dist.**, Gorakpur, **252-32**.

**moghulensis.****BOMBAY DECCAN.**

**Sholapur Dist.**, Kurduwadi, **349-31**.

**CHOTA NAGPUR.**

**Ranchi Dist.**, Kamdera, **259-32**.

**HYDERABAD (NORTH).**

**Aurangabad Dist.**, Aurangabad, **1-33**.

**KONKAN.**

**Savantvadi State**, **10-32, 12-32**.

**pallidus.****BENGAL.**

**Birbhum Dist.**, Sriniketan, near Surul, **142-32, 143-32**.

**BIHAR.**

**Darbhanga Dist.**, Madhubani, **242-32, 249-32, 274-32, 3-33, 22-33**; **Muzaffarpur Dist.**, Muzaffarpur, **333-31**.

**BOMBAY DECCAN.**

**Ahmednagar Dist.**, Ahmednagar, **302-31**; **Poona Dist.**, Poona, **68-31**.

**BURMA (UPPER).**

**Mandalay Dist.**, Maymyo, **339-31**.

CENTRAL INDIA (EAST).

**Bundelkand Agency, Nowgong, 360-31, 15-32.**

CHOTA NAGPUR.

**Palamau Dist., Barwadih, 198-32; Ranchi Dist., Kamdera, 259-32.**

KONKAN.

**Savantvadi State, 363-31, 10-32, 12-32, 32-32.**

MADRAS COAST (NORTH).

**Vizagapatam Dist., Govindpalli, 175-32, Mattili, 175-32, Pujariguda, 175-32.**

PUNJAB (EAST & NORTH).

**Ambala Dist., Ambala, 364-31; Karnal Dist., Barauta, 64-33, Budha Khera, 63-33, Darar, 60-33, Gagsina, 69-33, Ghogripur, 57-33, Indri 56-33, Jhanjari, 66-33, Kambohpora, 61-33, Karnal, 177-31, 186-32, 59-33, etc., Kunjpura, 58-33, Munak, 67-33, Rambha, 70-33, Saidpura, 55-33, Shahpur, 65-33, Taraori, 62-33; Patiala State, Badshahpura, 158-32, Narnaul, 158-32, Pinjaur, 283-32, Rajpura, 158-32, Sutariana, 158-32.**

UNITED PROVINCES (WEST).

**Aligarh Dist., Aligarh, 229-32; Barielly Dist., Barielly, 289-32; Saharanpur Dist., Roorkee, 231-32.**

*philippinensis.*

ASSAM.

**Goalpara Dist., Kachugaon, 229-31; Lakhimpur Dist., Jeypore, 128-31; Sylhet Dist., Sylhet, 194-32, 218-32.**

BENGAL.

**Birbhum Dist., Sriniketan, near Surul, 142-32, 143-32.**

BOMBAY DECCAN.

**Sholapur Dist., Kurduwadi, 349-31.**

MADRAS COAST (NORTH).

**Vizagapatam Dist., Govindpalli, 175-32.**

*pulcherrimus.*

GUJARAT.

**Baroda State, Baroda, 18-32.**

**NORTH WEST FRONTIER PROVINCE.**

**Peshawar Dist., Landikotal, 131-32.**

**PUNJAB (EAST & NORTH).**

**Karnal Dist., Indri, 56-33, Kambohpora, 61-33, Kunjpura, 58-33, Saidpura, 55-33, Taraori, 62-33; Jhelum Dist., Jhelum, 133-31; Patiala State, Badshahpura, 158-32, Rajpura, 158-32, Sutariana, 158-32.**

**RAJPUTANA (EAST).**

**Ajmer-Merwara Province, Ajmer, 96-32.**

**UNITED PROVINCES (WEST).**

**Saharanpur Dist., Roorkee, 259-31.**

**WAZIRISTAN.**

**Bannu Area, Mir Ali, 62-32.**

**ramsayi.**

**ASSAM.**

**Sylhet Dist., Sylhet, 194-32.**

**BENGAL.**

**Birbhum Dist., Sriniketan, near Surul, 143-32.**

**BIHAR.**

**Darbhangha Dist., Madhubani, 249-32, 274-32, 3-33.**

**splendidus (maculipalpis var. indiensis).**

**BENGAL.**

**Jalpaiguri Dist., Chuapara, 282-32; Nadia Dist., Birnagar, 57-32.**

**BOMBAY DECCAN.**

**Nasik Dist., Deolali, 2-31,**

**BURMA (LOWER).**

**Hanthawaddy Dist., Mingaladon, 161-32.**

**CENTRAL PROVINCES (WEST).**

**Nagpur Dist., Kamptee, 11-33.**

**CHOTA NAGPUR.**

**Manbhum Dist.,** Bhaga (Jherria Coal-fields), **47-31; Ranchi Dist.,** Kamdera, **259-32.**

**MADRAS COAST (NORTH).**

**Vizagapatam Dist.,** Boipariguda, **175-32, Govindpalli, 175-32, Jeypore Town, 179-32, Pottangi, 175-32.**

**NORTH WEST FRONTIER PROVINCE.**

**Peshawar Dist.,** Dargai, **94-31.**

**PUNJAB (EAST & NORTH).**

**Karnal Dist.,** Barauta, **64-33, Darar, 60-33, Gagsina, 69-33, Ghogripur, 57-33, Indri, 56-33, Munak, 67-33, Saidpura, 55-33, Taraori, 62-33.**

**stephensi.**

**BALUCHISTAN.**

**Quetta-Pishin Dist.,** Tabini Plateau, 7,150 ft., **72-31.**

**BOMBAY DECCAN.**

**Poona Dist.,** Lonavla, **42-31; Sholapur Dist.,** Bhamburdi, **34-31, Khudus, 34-31, Malaoh, 34-31.**

**CENTRAL INDIA (EAST).**

**Bundelkand Agency, Nowgong, 242-31.**

**CENTRAL PROVINCES (WEST).**

**Jubbulpore Dist.,** Jubbulpore, **29-31; Nagpur Dist.,** Kamptee, **34-32, 35-33; Saugor Dist.,** Bina, **181-32.**

**CHOTA NAGPUR.**

**Manbhum Dist.,** Bhaga (Jherria Coal-fields), **47-31.**

**GUJARAT.**

**Ahmedabad Dist.,** Ahmedabad, **114-32; Panch Mahals Dist.,** Freeland-ganj, **173-31.**

**HYDERABAD (SOUTH).**

**Atraf-i-Balda Dist.,** Hyderabad, **54-31.**

**MADRAS COAST (NORTH).**

**Vizagapatam Dist.,** Boipariguda, **175-32, Pottangi, 175-32.**

**NORTH WEST FRONTIER PROVINCE.****Chitral State**, Drosh, **168-32**; **Peshawar Dist.**, Risalpur, **113-31**.**PUNJAB (EAST & NORTH).****Karnal Dist.**, Barauta, **64-33**, Dadupur, **68-33**, Darar, **60-33**, Gagsina, **69-33**, Ghogripur, **57-33**, Indri, **56-33**, Jhanjari, **66-33**, Kambohpora, **61-33**, Kunjpura, **58-33**, Munak, **67-33**, Saidpura, **55-33**, Shahpur, **65-33**, Taraori, **62-33**; **Patiala State**, Narnaul, **158-32**, Pinjaur, **283-32**, Rajpura, **158-32**, Sutariana, **158-32**.**PUNJAB (SOUTH WEST).****Multan Dist.**, Multan, **8-31**, **58-32**, **69-32**, **83-32**.**RAJPUTANA (EAST).****Jaipur State**, Sambhar Town, **95-32**.**RAJPUTANA (WEST).****Sirohi State**, Mount Abu, **86-32**.**UNITED PROVINCES (EAST).****Gorakpur Dist.**, Gorakpur, **42-33**.**UNITED PROVINCES (WEST).****Meerut Dist.**, Babugarh, **272-31**.**subpictus.****ASSAM.****Goalpara Dist.**, Kachugaon, **229-31**.**BENGAL.****Birbhum Dist.**, Srimiketan, near Surul, **188-32**; **24-Parganas Dist.**, Falta, **240-31**.**BIHAR.****Darbhanga Dist.**, Madhubani, **242-32**, **249-32**, **3-33**, **22-33**; **Shahabad Dist.**, Deri-on-Sone, **213-32**.**BOMBAY DECCAN.****East Khandesh Dist.**, Bhusaval, **22-32**, **105-32**; **Sholapur Dist.**, Kurduwadi, **7-31**, Pandharpur, **34-31**.

**CENTRAL PROVINCES (WEST).**

**Jubbulpore Dist.,** Jubbulpore, **29-31, 9-32, 122-32, 172-32;** **Saugor Dist.,** Bina, **181-32.**

**CHOTA NAGPUR.**

**Manbhum Dist.,** Bhaga (Jherria Coal-fields), **47-31;** **Palamau Dist.,** Barwadih, **131-31, 198-32, 211-32, 241-32;** **Singbhum Dist.,** Naomundi, **36-31.**

**GUJARAT.**

**Panch Mahals Dist.,** Freelandganj, **173-31.**

**HYDERABAD (SOUTH).**

**Atraf-i-Balda Dist.,** Hyderabad, **54-31.**

**MADRAS COAST (NORTH).**

**Vizagapatam Dist.,** Jeypore Town, **179-32, Mattili, 175-32.**

**NORTH WEST FRONTIER PROVINCE.**

**Peshawar Dist.,** Cherat, **149-32, 159-32.**

**PUNJAB (EAST & NORTH).**

**Karnal Dist.,** Barauta, **64-33, Budha Khera, 63-33, Dadupur, 68-33, Darar, 60-33, Gagsina, 69-33, Ghogripur, 57-33, Indri, 56-33, Jhanjari, 66-33, Kambohpora, 61-33, Mohri, 130-32, Munak, 67-33, Rambha, 70-33, Saidpura, 55-33, Shahpur, 65-33, Taraori, 62-33;** **Patiala State, Badshahpura, 158-32, Mohindar Garh, 158-32, Narnaul, 158-32, Pinjaur, 70-31, 283-32, Rajpura, 158-32, Sutariana, 158-32.**

**RAJPUTANA (EAST).**

**Jaipur State, Sambhar Town, 95-32.**

**RAJPUTANA (WEST).**

**Sirohi State, Mount Abu, 86-32, 97-32.**

**UNITED PROVINCES (EAST).**

**Banda Dist.,** Manikpore, **172-32;** **Benares Dist.,** Benares, **246-31;** **Gorakpur Dist.,** Gorakpur, **203-32, 44-33.**

**UNITED PROVINCES (WEST).**

**Aligarh Dist.,** Aligarh, **229-32;** **Jhansi Dist.,** Jhansi, **325-31, 84-32;** **Meerut Dist.,** Babugarh, **204-31.**

**superpictus.****BALUCHISTAN.**

**Quetta-Pishin Dist.,** Tabini Plateau, 7,351 ft., **72-31.**

**WAZIRISTAN.**

**Derajat Area,** Wana, **124-31.**

**tessellatus.****ASSAM.**

**Lakhimpur Dist.,** Jeypore, **128-31; Sibsagar Dist.,** Cinnamara, **348-31.**

**BURMA (LOWER).**

**Hanthawaddy Dist.,** Mingaladon, **160-31.**

**CENTRAL PROVINCES (WEST).**

**Saugor Dist.,** Bina, **181-32.**

**CHOTA NAGPUR.**

**Ranchi Dist.,** Kamdera, **259-32.**

**GUJARAT.**

**Baroda State,** Baroda, **243-32.**

**HYDERABAD (SOUTH).**

**Atraf-i-Balda Dist.,** Hyderabad, **291-31.**

**MADRAS COAST (NORTH).**

**Vizagapatam Dist.,** Chatikona, **38-31,** Govindpalli, **175-32,** Pottangi **175-32.**

**theobaldi.****BOMBAY DECCAN.**

**Ahmednagar Dist.,** Ahmednagar, **332-31; Poona Dist.,** Lonavla, **42-31.**

**CENTRAL INDIA (EAST).**

**Bundelkand Agency,** Nowgong, **354-31.**

**CENTRAL PROVINCES (WEST).**

**Nagpur Dist.,** Kamptee, **4-33, 25-33, 35-33.**

**MADRAS COAST (NORTH).**

**Vizagapatam Dist.,** Govindpalli, **175-32.**

**ORISSA.**

**Kalahandi State,** Dabriguda, near Ampani, **67-32.**

**RAJPUTANA (WEST).**

**Sirohi State,** Mount Abu, **97-32.**

**UNITED PROVINCES (WEST).**

**Jhansi Dist.,** Jhansi, **325-31.**

**turkhudi.**

**BALUCHISTAN.**

**Quetta-Pishin Dist.,** Tabini Plateau, 7,351 ft., **72-31.**

**BOMBAY DECCAN.**

**Sholapur Dist.,** Bhamburdi, **34-31,** Khudus, **34-31,** Kurduwadi, **349-31,** Malaoli, **34-31,** Mandwe, **34-31.**

**CENTRAL PROVINCES (WEST).**

**Nagpur Dist.,** Kamptee, **35-33.**

**MYSORE.**

**Kadur Dist.,** Mudigere, **36-32.**

**NORTH WEST FRONTIER PROVINCE.**

**Peshawar Dist.,** Shahgai, **98-32.**

**PUNJAB (EAST & NORTH).**

**Karnal Dist.,** Karnal, **59-33.**

**SIND.**

**Larkana Dist.,** Dodai, **345-31.**

**WAZIRISTAN.**

**Derajat Area,** Wana, **172-31, 138-32.**

**umbrosus.**

**ASSAM.**

**Lakhimpur Dist.,** Margherita, **237-31; Sibsagar Dist.,** Cinnamara, **315-31, 234-32,**



**vagus.****ASSAM.**

**Kamrup Dist.**, Khanapara, **230-31**; **Lakhimpur Dist.**, Jeypore, **128-31**, Namsang, **206-31**; **Naga Hills Dist.**, Kohima, **202-32**; **Sibsagar Dist.**, Deopani Tea Estate, **180-31**; **Sylhet Dist.**, Sylhet, **194-32**.

**BENGAL.**

**Jalpaiguri Dist.**, Alipur Duar, **139-31**.

**BIHAR.**

**Darbhanga Dist.**, Madhubani, **242-32, 3-33, 22-33**.

**BOMBAY DECCAN.-**

**Poona Dist.**, Poona, **257-31**.

**CHOTA NAGPUR.**

**Palamau Dist.**, Barwadih, **198-32**.

**GUJARAT.**

**Baroda State**, Baroda, **18-32**.

**MADRAS COAST (NORTH).**

**Vizagapatam Dist.**, Govindpalli, **175-32**.

**varuna.****BENGAL.**

**Birbhum Dist.**, Sriniketan, near Surul, **142-32, 143-32**.

**BIHAR.**

**Darbhanga Dist.**, Madhubani, **3-33**.

**CHOTA NAGPUR.**

**Ranchi Dist.**, Kamdera, **259-32**.

**KONKAN**

**Savantvadi State**, **49-31, 56-31, 363-31, 10-32, 12-32, 32-32, 121-32**.

**MADRAS COAST (NORTH).**

**Vizagapatam Dist.**, Boipariguda, **175-32**, Govindpalli, **175-32**, Jeypore Town, **179-32**, Pottangi, **175-32**, Pujariguda, **175-32**.

**MYSORE.**

**Kadur Dist., Mudigere, 359-31, 36-32.**

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| COVELL, G. (1931) | .. | .. | The Distribution of Anopheline Mosquitoes in India and Ceylon: Additional Records, 1926-1930. <i>Records of the Malaria Survey of India</i> , 2, pp. 225-268. |



NOTE ON A MOSQUITO WITH THE POLLINIA OF AN *ORCHIS*  
ATTACHED TO ITS PROBOSCIS.

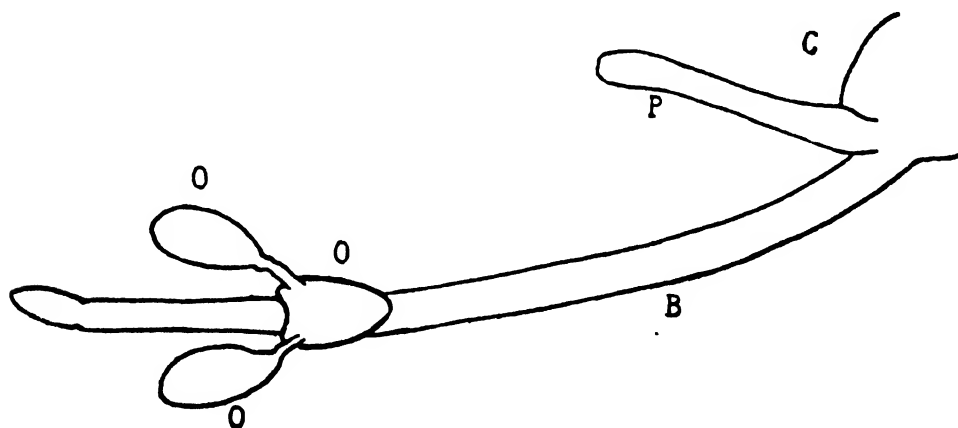
BY

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(*Entomologist to the Malaria Survey of India, Kasauli*),  
(*Indian Research Fund Association*).

[April 28, 1933.]

It seems worth while to record that there is a specimen of *Munsonia* (*Mansonioides*) *uniformis* (Theo.) in the Malaria Survey of India collection, Kasauli, with the pollinia of an *Orchis* attached to its proboscis. The accompanying outline camera-lucida sketch shows the appearance of the pollinia, and their position on the proboscis. The specimen is a female and was collected at Tando Bago, Hyderabad district, Sind, by Rai Sahib J. D. Baily, I.M.D., 2. x. 1928. The mesonotum is partly denuded, but the identification has been checked by examination of the hypopygium, which in this subgenus, shows well-marked specific differences in the females, as well as in the males.



Camera-lucida outline sketch of the proboscis of a female specimen of *Munsonia* (*Mansonioides*) *uniformis* (Theo.) with the pollinia of an *Orchis* attached. C = clypeus; P = palp; B = proboscis; O = pollinia.

Pollinia may often be found attached to the mouth parts of flower-frequenting insects in places where species of *Orchis* are common, but the occurrence does not seem to have been recorded previously in the case of mosquitoes. There are, however, numerous records of mosquitoes, both males and females, of both blood-sucking and non-blood-sucking forms, visiting flowers for the purpose of sucking honey.

## STUDIES IN IMMUNITY IN MALARIA.

### Part II.

#### *SUPERINFECTION WITH VARIOUS STRAINS OF MONKEY MALARIAL PARASITES.*

BY

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AND

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*(Malaria Survey of India, Kasauli.)*

*(Research under the Indian Research Fund Association.)*

It has been known for a long time that different strains, of what are apparently the same species of *Plasmodium*, may vary very considerably in their clinical effects on the infected animal host. It is only in recent years, however, that the great importance of superinfection, by the same or different strains of these parasites, has begun to be fully recognized in relation to the epidemiology and the immunology of human malaria.

The effects of superinfection in malaria have been the subject of much study in the past. This work has been confined mainly to experiments with bird malaria. This was chiefly because there was no other experimental animal easily available, which was susceptible to plasmodial infections. With the advent of malarial therapy for various mental diseases, experimental work has been extended to man. In the latter case, however, the scope of these investigations has been limited by the fact that infections with the more virulent strains cannot be allowed to run to their natural conclusions without therapeutic intervention.

The isolation of five strains of *P. knowlesi* and one of *P. inui* var. *cynomolgi* from the monkey, *Simulans irus*, has opened up a wide field for the investigation of the effects of superinfection with different strains of malarial parasites (Sinton and Mulligan, 1933). These simian parasites are especially suitable, because normal susceptible hosts (i.e., *S. rhesus*) are easily available. The

fact that the infections are being studied in a Primate host should make comparisons with human malarial infections more valuable than in the case of the disease in birds.\*

It is not proposed in this paper to discuss at any great length the deductions which might be made from the evidence available, in relation to superinfection with different strains of malarial parasite. It is felt that this subject can be dealt with better when an even larger series of experiments has been carried out. A summary of previous work seems, however, essential for the proper understanding of the subject.

### PREVIOUS WORK ON BIRD MALARIA.

A large amount of work on superinfection with the various parasites found in malarial infections in birds has been done in the last thirty years. Most of this work seems to have been carried out by reinfection with the same strain of parasite, but Gingrich (1932) has recently investigated the effects of superinfection with different strains, and cross-immunisation with different species, of bird malarial parasites.

#### (1) SUPERINFECTION WITH A HOMOLOGOUS STRAIN.

The work which has been done on superinfection with the same strains of parasite has been summarized by Gingrich (1932) as follows:—

'It has been demonstrated by a number of investigators that when a bird has a latent or chronic malarial infection it is refractory to re-inoculation of the same strain or species. Koch (1899) believed that birds were immune as a result of complete recovery from infection, but von Wasielewski (1902) observed that the initial acute infection was generally followed by a latent or chronic infection continuing for an indefinite length of time during which the birds were immune to superinfection. Then Moldovan (1912) found that a bird was immune only as long as the latent or chronic infection persisted, and upon recovery a bird became as susceptible as normal birds. Whitmore (1918) found that infection continued in one bird for 29 months and also demonstrated immunity to superinfection in two birds which were known to be harbouring chronic infection of 4 months' and 20 months' duration, respectively, after their first inoculations. Sergeant and Hempl (1917) reported that the immunity to superinfection lasted two and a half years in 4 out of 5 birds and Kikuth and Tropp (1927) found 24 out of 25 birds immune to superinfection (a relapse having occurred in one bird on the day following the re-inoculation) from 18 days to 5 months after their initial infections. Mazza (1924) found that

---

\* These infections have already been found invaluable for teaching purposes. They have enabled our students to study individually, and make permanent preparations of, the various stages of the cycle of sporogony (exflagellation, ookinetes, oöcysts and sporozoites). *P. inui* var. *cynomolgi*, on account of the marked stippling of the infested cells, has also proved very useful for making preparations upon which students can try various staining methods and thus perfect their technique.

infection persisted in one bird for a period of 4 years and 2 months and also that two other birds were uninfected (demonstrated by negative inoculation, "isodiagnosis") 3 years and 3 months after their initial infections and were then susceptible to a second infection. Recently Manwell (1930) reported a number of complete cures from treatment with plasmochin and with quinine, and found the birds susceptible to reinfection. The relationship between the immunity to superinfection and the persisting latent infection has thus been well established so that superinfection is now being used to demonstrate the presence or absence of a latent infection, as suggested by Sergeant (1920)\*.

A somewhat similar summary has been given by Taliaferro and Taliaferro (1929) of the earlier work on this subject.

Manwell (1929) found that a strain of *P. relictum* (*P. inconstans*) caused no superinfection when injected into a bird having a latent infection with another strain of the same parasite. The differentiation of several species of bird Plasmodia has enabled Gingrich (1932) to carry out superinfection experiments with *P. elongatum*, *P. cathemerium*, *P. relictum* and *P. rouxi*. He reports that a latent or chronic infection with *P. elongatum*, *P. cathemerium* or *P. relictum* is associated with an effective immunity to superinfection with the same strain of parasite. On the other hand, *P. rouxi* confers a relatively inefficient immunity to superinfection with the same strain. Sergeant, Sergeant and Catanei (1932) also found that a chronic infection with *P. elongatum* protected against superinfection with the same strain of parasite.

A case of complete recovery from an infection with *P. elongatum* with subsequent susceptibility to reinfection is also reported by Gingrich. This he considers to be significant, in supporting the theory that the acquired immunity to malarial infections of birds is operative only as long as the latent or chronic infection persists.

Gingrich (1932) believes that 'the acquired immunity to each of the four species is primarily a specific reaction and a non-specific factor may play a minor rôle'.

Most, if not all, of the above investigations appear to have been confined to attempts to superinfect birds already showing a latent or chronic infection with the same strain of parasite.

## (2) SUPERINFECTION WITH A HETEROLOGOUS STRAIN OF PARASITE.

Kikuth (1931) reported a cross-immunity between a strain of *P. inconstans* and one of *P. praecox*. Gingrich (1932) believes, from his study of these strains, that these two apparently different species were both *P. relictum*.

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\*In the early work with the malarial parasites of birds, although several workers suspected that the parasites used in different countries probably belonged to several different species of *Plasmodium*, it was not until 1927 that a number of distinct species was definitely recognized. Hartman (1927) separated three species, *P. praecox*, *P. cathemerium* and *P. inconstans*. Since then Huff (1930) has described *P. elongatum*, Sergeant, Sergeant and Catanei (1928) *P. rouxi*, Kikuth (1931) *P. circumflexum*, and Russell (1932) *P. capistrani*. The synonymy of several of these species has since been studied by Gingrich (1932).



Gingrich (1932) seems to be the only worker who has studied the effects of superinfection with a number of different strains of the same parasite. This worker reports that a latent or chronic infection of any of the five strains of *P. relictum* used by him, is associated with an effective immunity to superinfection with any of the five strains of the same species.

### (3) CROSS-IMMUNITY WITH DIFFERENT SPECIES OF PARASITE.

Hartman (1927), in his work with *P. relictum* (*P. inconstans*), *P. cathemerium* and *P. elongatum* (*P. praecox*),\* found that 'birds which had had an infection with one species were as easy to infect with either or both the other species as were birds which had never been infected'.

Manwell (1929) reports that *P. elongatum* (*P. praecox*) caused very severe infections when inoculated into birds infected with *P. relictum* ('Whitmore strain').\*

Sargent *et al.* (1929; 1931) found that inoculation with *P. relictum* produced sub-acute infections in two birds infected with *P. cathemerium*; their results from the cross-inoculation of *P. relictum* and *P. rouxi* were variable. The same workers found that *P. cathemerium* produced fatal results in two birds infected with *P. rouxi* and also that the former species produced infections in birds carrying *P. relictum*.\* At a later date, these workers (1932) found that a chronic infection with *P. elongatum* conferred no cross-immunity against a superinfection with *P. relictum*, and *vice versa*.

Kikuth (1931) reports a partial cross-immunity between *P. relictum* and infections with *P. cathemerium*, but no cross-immunity between *P. elongatum* and either *P. cathemerium* or *P. relictum*. On the other hand, *P. cathemerium* produced an acute infection when superimposed on *P. relictum*. Kikuth (1931) also found that *P. circumflexum* produced cross-immunity with *P. cathemerium* and *P. relictum*, but there was no cross-immunity between the first species and *P. elongatum*.\*

Gingrich (1932) has done a large amount of work on this subject. He reports :—

(a) A latent infection with *P. cathemerium* is associated with a partial cross-immunity to *P. relictum* and *P. rouxi*, but no cross-immunity to *P. elongatum*.

(b) A latent infection with *P. relictum* is associated with an effective cross-immunity to *P. cathemerium*, but none to *P. rouxi* or to *P. elongatum*.

(c) A chronic infection with *P. rouxi* is associated with a partial cross-immunity to *P. cathemerium*, but none to *P. relictum* or to *P. elongatum*.

(d) A latent infection with *P. elongatum* is associated with no cross-immunity to any of the other three species.

Gingrich (1932) concludes that 'the occurrence of cross-immunity between two species suggests a measure of similarity of their antigenic properties (using

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\*The synonymy of the different species is that given by Gingrich (1932).

the term "antigenic" in the broader sense, meaning the property of exciting specific immune reaction) '.

#### SUMMARY.

The subject of superinfection and cross-immunity with several strains of the different species of avian Plasmodia, has been studied by many workers. The number of experiments made and the variety of strains used, have in most instances been few. Further work is needed to confirm many of the findings reported.

The evidence available suggests that—

(a) The presence of a latent or chronic infection with *P. relictum* confers an effective immunity against superinfection with either homologous or heterologous strains of the same species of parasite.

(b) Latent or chronic infections with either *P. elongatum* or *P. cathemerium* are associated with an effective immunity against superinfection with a homologous strain of the same species of parasite.

(c) A latent or chronic infection with *P. rouxi* confers a relatively inefficient immunity to superinfection with the same strain of parasite.

(d) The investigations into the cross-immunity produced by one species of avian *Plasmodium* against superinfection with another species, indicate that

- (i) Latent infections with *P. relictum* may, in some instances, confer varying degrees of immunity against superinfection with *P. cathemerium*, but not against *P. elongatum* or *P. rouxi*.
- (ii) Latent infections with *P. cathemerium* may, in some cases, confer slight immunity against superinfection with *P. relictum* or *P. rouxi*, but not against *P. elongatum*.
- (iii) Latent infections with *P. elongatum* confer no immunity against superinfection with either *P. relictum*, *P. cathemerium* or *P. rouxi*.
- (iv) Latent infections with *P. rouxi* confer no immunity against superinfection with *P. relictum* or *P. elongatum*, but partial immunity against *P. cathemerium*.
- (v) Latent infections with *P. circumflexum* may confer partial immunity against *P. relictum* or *P. cathemerium*, but not against *P. elongatum*.

#### PREVIOUS WORK ON HUMAN MALARIA.

Before the question of superinfection in human malaria can be discussed, the evidence in favour of the occurrence of different strains of the various species of human Plasmodia must be considered. Such strains have been distinguished by variations in the intensity or virulence of their clinical effects, in their power to produce immunity or tolerance\* to the effects of

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\* The 'immunity' in malaria is rather of the nature of a tolerance to the toxic effects of the infection, and its persistence often appears to depend upon the continued presence of parasites in the body.

superinfection with the same or other strains of the same species, and in their reactions to therapeutic agents.

In some instances variations have been recorded between strains of the same *Plasmodium* originally derived from different areas. There is also evidence to suggest that, in some cases, the virulence of the same strain has changed during experimental passage through either the insect or animal hosts. Whether the varied degrees of virulence found under natural conditions in parasites from different localities are due to such passages, during a comparatively short period of time, or whether they are more stable properties developed during countless generations is uncertain.\*

#### \ (1) STRAINS OF HUMAN MALARIAL PARASITES.

##### (a) Natural variations in the virulence of different strains.

Most of the experimental work in regard to this subject relates to *P. vivax*. Within the last few years experimental infections with strains of *P. falciparum* showing different degrees of virulence have been reported, but such variations in *P. malariae* do not appear to have been studied.

Bates (1912) does not believe that variations in the virulence of strains occur in different countries. Yorke (1925) thinks that 'observations made on War cases did not support this hypothesis (i.e., of different strains), as the vast majority of patients from such different regions as Gallipoli, Salonica, Mesopotamia, India and Africa relapsed'. He also states that the strain of parasite had little or no effect on the causation of relapse, although in the theory of cure formulated by Yorke and Macfie (1924) the development of immune-body resistant strains is mentioned.

Marchoux (1922) thinks that there is a multiplicity of strains of the three common species of human Plasmodia. This, he considers, explains why Senegal negroes, immune to malaria in their own country, succumb to the disease when taken to Dahomey. Leger and Nogue (1923) have also brought forward epidemiological evidence in support of the view that the inhabitants of an area may become tolerant to local strains of parasite, yet at the same time be susceptible to the pathogenic effects of strains present in other localities. The Malaria Commission of the League of Nations (1925), as a result of their tour

\* The apparent increase in the intensity of the clinical manifestations of malaria during fulminant epidemics, and in connection with tropical aggregations of labour, suggests that a comparatively rapid change in virulence may occur and may be an important factor in the causation of such conditions. On the other hand, Gill (1928) considers that changes in virulence play only a minor, if any, part in the genesis of epidemics. He thinks that the greater severity of the disease is due to a larger dosage of sporozoites. In the severe malaria of tropical aggregations of labour, the possibility of the introduction of new strains of parasite cannot be excluded. It is possible that all these factors may play parts of greater or lesser importance (vide Sinton, 1933).

in the malarious countries of Europe, consider that the interchange of plasmodia, resulting from the mass movements of people, has not been sufficiently stressed as a factor of primary importance in the causation of the greatly enhanced malarial incidence, which occurred in these countries after the War. Boeckh (1925) also believes that immunity to malaria varies with different strains of the same parasite.

Sinton (1931) has discussed, in some detail, the evidence in favour of the occurrence of different strains of the human malarial parasites, and of variations in their virulence. The probable importance of infection and superinfection with such strains in the production of both clinical and permanent cure, by either natural or therapeutic means, was pointed out. James (1932), Schulemann (1932) and Swellengrebel (1932) also comment on the importance of strain, in relation to tests for the evaluation of the therapeutic efficiency or activity of different drugs.

#### (i) *Strains of P. vivax.*

Observations on therapeutic infections with *P. vivax* suggest that the difference between apparent resistance to parasitic infection (or its clinical manifestation), and infection with marked clinical symptoms, is only a matter of degree, all stages intermediate between these two extremes being found.

Kirschbaum (1927) reports that in therapeutic malaria he was unable to detect any differences in the virulence of the strains of *P. vivax* used by him, although the geographical origin of these strains was very diverse. He attributes the different clinical effects observed to individual differences in the hosts. On the other hand, the observations of some workers (Rudolf, 1924; Pijper and Russell, 1925; Bunker and Kirby, 1925; Lilly, 1925) would indicate that the virulence of different strains of this parasite may vary very considerably, as judged by the severity of the clinical symptoms produced.

Swellengrebel (1932) notes marked differences between the indigenous Dutch strains of *P. vivax* and the Madagascar strain used by James in England. These differences were apparent in the clinical effects, in the duration of the incubation period after mosquito bite, in the power to infect *A. maculipennis*, and in the reaction of the infection to treatment with various drugs. Kortweg (1931) had already suggested that these two strains might be different, because of their different clinical manifestations.

#### (ii) *Strains of P. falciparum.*

James (1932) states that 'the biological property of virulence varies not only with different species of a parasite but with different geographical strains of the same species'. He finds that the Indian strains of *P. falciparum* used in his work are much less virulent than the Italian ones. He also points out

that Grassi, as long ago as 1900, recognized two distinct clinical varieties of malignant tertian malaria in Italy, a mild one (*mitis*) and a severe one (*immitis*).\*

James, Nicol and Shute (1932) studied eight strains of *P. falciparum* (two from India, three from Rome, one from Sardinia and two from West Africa). They 'believe that a biological study of these strains yields definite proof that within the same species there are various geographical races which, while not being morphologically different, can be recognized as being distinct by their clinical virulence, immunological reactions and other biological properties'.

Watson (1932) also suggests that there may be a specially virulent strain of *P. falciparum* among the aboriginal tribes in the Jeypore Hill Tracts of Madras, which may be responsible for blackwater fever in that area.†

#### (b) Changes in the virulence of the same strain of parasite.

It is well known that certain bacteria lose their virulence after cultivation on artificial media, and that this virulence can be re-established by passage through susceptible animals. Although it is very unwise to place too much reliance on the behaviour of bacteria, which belong to the vegetable kingdom, as compared with protozoa, which are of animal origin, such a possibility must be considered. This is more especially the case, since it has been found that the virulence of some of the other pathogenic protozoa has been enhanced by animal passage.

In the case of direct blood passage of *P. vivax* through the human host, Reese and Peter (1924), Yorke and Macfie (1924) and Marginescu (1930) state that they have found no increase in clinical virulence after repeated passage. Boyd (1925) records similar findings with bird malaria. On the other hand, James (1924a) reported that the strain which he was using at that time 'has become more "virulent" than the benign tertian strains commonly encountered in the tropics'. He believes (James, 1924) that 'this severity is a consequence in part of an increased activity and virulence resulting from

\* James, Nicol and Shute (1932) were unable to find any morphological differences between the parasites of the Indian and the Italian 'strains' of *P. falciparum* used by them. Although we believe it to be certain that strains of *P. falciparum* with very varied virulence exist, yet, we think that in considering this species of parasite, one must remember it is by no means proven that all the parasites, which are at present grouped under this name, belong to the same species. It may be that some of the wide variations in virulence recorded are due to differences in species rather than in strains only. The fact that James, Nicol and Shute (1932) failed to infect *A. matulipennis* with the Indian strain, but succeeded easily with the Italian ones, must not be overlooked in this connection.

† The possibility that blackwater fever may be due to a different species of 'malignant tertian parasite' has been suggested by many different workers (Sinton, 1927).

the cultivation of the parasite by direct passage through human hosts'. Hermann (1924), MacBride and Templeton (1924) and Bunker and Kirby (1925) consider that an enhanced virulence has developed in the strains used by them. These observations suggest that increased rapidity of passage through the animal host cannot be neglected, in our present state of knowledge, as a possible factor in causing changes in the clinical virulence of the malarial parasite.

Engel (1918) has put forward the theory that the climate, in which the anopheline host lives, may influence the toxic characters of *P. vivax*. It is well known that certain protozoal strains renew their vitality by conjugation, so it is possible that the sexual cycle of the malarial parasite in the insect host may rejuvenate or increase the vitality of the parasite. In the case of trypanosomes, however, Duke (1923, 1928) thinks that epidemic sleeping sickness is due rather to rapid direct mechanical passage through a series of animal hosts, and that passage of the trypanosome through the tsetse fly tends to lower its virulence. In this trypanosome, however, the evidence of a sexual cycle in the insect host still requires confirmation.

James (1924) at one time, thought that the increased virulence, developed by his strains of *P. vivax* after repeated passage through man, might possibly be lost by passage of the strain through the insect host. This did not appear to take place, and any change noted seemed to be rather in the nature of an increase than a diminution of virulence. At a later date, this worker (James, 1931), from observations upon another strain of *P. vivax* which he obtained in 1925, found that 'it does not seem to have undergone any change of virulence' after mosquito passage over a period of 5½ years. Rudolf (1927) reports that patients, refractory to one strain of parasite by direct blood inoculation, could be infected by the same strain after passage through the mosquito.

Watson (1932) also concludes that, while Malays may have acquired a considerable immunity to the local strains in their own area, yet the virulence of these strains may be so raised by passage through mosquitoes and Indian emigrants that the immunity of the local inhabitants in Malaya is no longer able to counteract the clinical effects of fresh infection.

Very different results have been reported in the effects of treatment in the production of a permanent cure of malaria induced by blood inoculation, as compared with those infections transmitted by mosquito bite. This would also suggest that passage through the insect host may modify considerably the cure-resistant properties of these parasites, at least of some strains of *P. vivax*.

The multiple infections which occurred during the War must have resulted in a very rapid passage of the parasite through a series of insect and human hosts in many instances. The evidence summarized above suggests that such passages cannot be neglected as possible factors in tending to raise the virulence of the parasite, and in causing the development of a more toxic or a more resistant strain of parasite than that originally present (Sinton, 1931).

(c) Variations in the reactions of different strains to therapeutic measures.

Many workers believe that quinine-resistant\* strains of the malarial parasites exist. The administration of 'prophylactic quinine' is supposed to play an important rôle in the production of such strains. One of the explanations put forward to account for the severe type of malaria seen during the Great War, was that strains of this nature had been introduced by troops from other localities. Plehn (1927) reports such a strain of *P. falciparum*, which persisted during three passages through patients being treated for general paralysis of the insane, but found that while the parasites resisted quinine, this resistance had no relationship to the toxicity of the parasite.† Arguing from his observations, Plehn (1927) considers that the severe malaria of the War can be explained by the development of such resistant strains, which maintained their peculiarity through several passages. He suggests that the quinine-sensitive strains would be killed off before the formation of gametocytes, by the intensive quininization in force, while the resistant strains would survive and produce gametocytes. They would thus be spread by the mosquito and become the predominant strain. Ziemann (1920) also thinks that malarial parasites may be divided into avirulent, virulent and intermediate forms, according to their degree of resistance to quinine medication. Wenyon *et al.* (1921) have put forward the hypothesis that the failure of quinine prophylaxis during the War may have been due either to certain sporozoites, or the young forms arising immediately from them, being resistant to quinine.

If such quinine-resistant forms exist in India, as judged by the failure of quinine properly administered to cure clinical symptoms and to cause a disappearance of parasites from the peripheral blood, they must be extremely rare, at least in Northern India. An exhaustive search for such strains among nearly 4,000 cases of naturally acquired malaria, carefully investigated during the last 10 years, has failed to reveal a single resistant case. This was so, in spite of the fact that more than half of the patients had previously been subjected to many and prolonged courses of quinine treatment. Bates (1912), from his work in Panama, also concluded that varieties of malarial parasite inherently resistant to quinine did not occur, nor were 'quinine-fast' strains produced.

These observations are of great interest in conjunction with the work of James (1932). This author found that the severe Rome strain of *P. falciparum*

\*The term 'quinine-resistant' is usually applied to denote those cases in which it is reported that the parasites in the peripheral blood, or the fever, persists in spite of adequate doses of quinine. As will be noticed below, some workers have used this term to denote also those cases in which a clinical cure is produced by treatment, but which relapse at a later date after this has terminated. These two conditions are quite distinct.

†Sergent and Sergent (1921) have also reported a quinine-resistant strain of avian *Plasmodium*, which developed in their birds in Algeria. This strain regained its normal characters after several passages.

used by him, required ten times as much quinine as was required to control infections produced by a mild Indian strain. The Indian strain was permanently cured by quinine with comparative ease. On the other hand, the Rome strain continued to relapse during several months, in spite of many courses of treatment with quinine, alone or in combination with plasmoquine. The latter infections were found to be quickly cured by atebirin.

James (1932) states that Kortweg found that neosalvarsan has a powerful action on the severe Madagascar strain of *P. vivax* employed by James, but a very weak action on the mild indigenous Dutch strain. Swellengrebel (1932) reports other observations, chiefly those of Kortweg and Piebenga, on the chemotherapy of these two strains. Apparently the relapse rate with the Dutch strain, after ordinary quinine treatment, does not exceed 50 per cent, as compared with a relapse rate of 100 per cent with the Madagascar strain after similar treatment.

James (1932) points out that 'the remarkable contrast brought out by therapeutic tests raises the question whether, from the chemotherapeutic point of view, the biological property of susceptibility of a parasite to the action of drugs may not be more important than its morphological characters—strains may be more important than species'.\*

## (2) SUPERINFECTION IN HUMAN MALARIA.

The immunity which develops after an infection with any particular strain of malarial parasite is rather of the nature of a 'tolerance' to the clinical effects produced by that plasmodial infection than a true immunity.

The work of James (1931) (*vide infra* 'Superinfection with an homologous strain of *P. vivax*') suggests that some degree of tolerance is acquired comparatively soon in untreated infections of *P. vivax*. The formation of a complete tolerance only occurs after 'spontaneous recovery', and apparently takes a number of months to develop. James, Nicol and Shute (1932) mention the observations of Koch, Marchiafava and others to the effect that tolerance to an infection with *P. falciparum* is so quickly acquired that, as a rule, the risk of pernicious symptoms is confined to the primary attack.

James (1931) believes that, with infections due to *P. vivax*, if the disease be allowed to continue untreated for a considerable period, the tolerance which is developed materially helps the therapeutic action of quinine. On the other hand, quinine given in the absence of such tolerance appears less effective. Early treatment would, therefore, appear to diminish the amount of immunity produced by an infecting strain. Ciuca (1931), also, found that such patients when treated with quinine, did not acquire so much resistance to subsequent reinfection as those in whom the infection was allowed to run to spontaneous recovery. Of patients treated with quinine in the primary attack, 32 per cent

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\* *Vide* foot-note on p. 536.



could be reinfected, while only 15 per cent of those who had recovered spontaneously were susceptible to reinfection.

The evidence at present available suggests that the duration of this tolerance is largely dependent upon the continued presence of the infecting organisms in the body. For what length of time and in what degree of efficiency this tolerance persists in the human subject after the infection has been cured, has not been definitely proven. There is, however, evidence suggesting that in the absence of reinfection after cure the tolerance rapidly diminishes.

Thomson (1924) noticed that natives, tolerant to malaria in highly endemic areas of Rhodesia, quickly lost their immunity by sojourn in non-malarious localities. Kligler (1930) came to the conclusion that, in so far as children are concerned, no immunity to reinfection existed after cure with quinine, but that in adults there was a heightened resistance.\* Djaparidse (1932) considers on epidemiological grounds that infections with *P. vivax* may persist in the body for years, and, so long as the parasite remains, protection is present against superinfection with this species of parasite. On the other hand, in the case of *P. falciparum*, infections are of short duration and thus any protection against superinfection is quickly lost.

#### (a) Superinfection with a homologous strain of *P. vivax*.

It is now definitely established, chiefly through the work of James and his colleagues on therapeutic malaria, that a definite resistance to reinfection can be established against a strain of *P. vivax*. Superinfection with a homologous strain in the presence of such tolerance causes few or no clinical symptoms, and slight, if any, increase in the number of parasites in the peripheral blood. The degree of tolerance to the 'toxic' effects of the infecting organism is probably relative only, and may vary with conditions affecting the health of the host. If such conditions cause a lowering of the tolerance, a relapse, either febrile or parasitic or both, may occur.

James and Shute (1926) concluded from their work that 'one attack of malaria (induced either by blood inoculation or mosquito bites) due to a strain of *P. vivax* does not confer an immunity against a second infection with the same strain', but point out that 'the clinical character of the second infection is quite different from the primary infection'. Plehn (1926) also records tolerance to superinfection with the same strain of this parasite.

Ciuca, Ballif and Viéru (1928) and Ciuca, Ballif, Viéru and Stirbu (1929) found that patients infected with *P. vivax* on one or more occasions may develop an acquired immunity to further infections with the same parasite.

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\* Apart from the findings of James (1931) and of Ciuca (1931) mentioned above, this may only mean that the children during their short lives have developed an immunity to one or two local strains only, and have been reinfected with a different or heterologous strain to which they have little or no tolerance. On the other hand, the adults have probably developed an immunity to a much larger number of strains, so the chances of reinfection with a heterologous strain become much less.

This immunity was not, however, effective against *P. falciparum*, and was only slightly so against *P. malariae*. Milani and Cubani (1931) also state that, with *P. vivax*, immunity increases with successive re-inoculations; the greater the number of re-inoculations the smaller is the chance of being able to produce a reinfection.

James (1931) has discussed at some length the question of tolerance and 'immunity' in benign tertian malaria. He concludes that 'at any time during the period prior to the recurrence\* the patient can be given a second attack (and in some cases a third) by reinfection with the same parasite either by mosquito bites or by direct blood inoculation, but this attack is less severe and less prolonged than the primary attack. On the other hand, after there has been a recurrence and "spontaneous recovery", endeavours to bring on a fresh attack by reinfection with the same parasite fail. We have not as yet been able to ascertain whether this means a complete disappearance of parasites from the body, but in our experience these patients are completely proof against reinfection'.

'In our experience this condition of immunity occurs only when each attack or relapse\* has been allowed to run its course for some time without quinine treatment. It is most marked in patients who have had "spontaneous recoveries" from a recurrence' (James, 1931).

Boyd and Stratman-Thomas (1933) also found that superinfection with the same strain of *P. vivax* as that which caused the initial attack, produced no clinical symptoms.

### (b) Superinfection with a heterologous strain of *P. vivax*.

This condition of tolerance to superinfection with the same strain of *P. vivax* does not, however, hold if the superinfection be caused by other strains of the same species of parasite.

Patients who have proved refractory to infection with one strain of this parasite have been found susceptible to another (Nicole and Steel, 1926), or even to the same strain after passage through the mosquito (Rudolf, 1927). Rudolf (1927) suggests, as the result of his work, that 'the question of the degree of immunity produced by artificially-inoculated malaria varies with the strain of parasite employed'. James and Shute (1926) managed, with a

\* With regard to benign tertian infections, James (1931) states that 'for our own purposes, and quite arbitrarily, we distinguish between the returns of fever and parasites which may follow recovery from a primary attack, thus:

*Recrudescence*.—A return of fever and parasites at any time within eight weeks after recovery from the primary attack.

*Relapse*.—A return of fever and parasites later than eight weeks, but earlier than twenty-four weeks after recovery from the primary attack.

*Recurrence*.—A return of fever and parasites later than twenty-four weeks after recovery from the primary attack. This means as a rule later than twenty-six weeks after the date of primary infection.

different strain of *P. vivax*, to infect, by means of mosquitoes, some cases which had previously been found resistant to blood inoculations with another strain. These workers draw the conclusion that 'several artificial inoculations, at intervals, by the direct-blood method of one strain of *P. vivax*, do not confer an immunity against mosquito infection with another *vivax* strain'.

James (1931) states that 'a surprising finding is that complete immunity to reinfection with one strain of *P. vivax* does not confer protection (or only confers partial protection) against another strain of the same species'.

Boyd and Stratman-Thomas (1933) investigated the effects of superinfection by means of three different strains of *P. vivax* obtained from widely separated localities within a 25-mile radius. The infections were transmitted by mosquito bites. These workers found that superinfection with the same strain as that of the initial attack produced no clinical symptoms. On the other hand, superinfection with a different strain causes an attack of malarial fever. They conclude that 'a person infected with benign tertian malaria acquires a homologous but not a heterologous tolerance to *P. vivax*'.

These results suggest that, while a certain degree of 'species tolerance' may be developed, this is not sufficient to counteract completely the virulence of a superinfection by another strain of the same species of parasite.

### (c) Superinfection with *P. falciparum*.

Plehn (1926) reports that with the aid of quinine medication and repeated superinfection, he was able to establish a tolerance to superinfection with the same strain of *P. falciparum*.

James, Nicol and Shute (1932) infected a patient with an Indian strain of *P. falciparum* by blood inoculation. The primary attack and subsequent relapses, which occurred within 6 weeks, were controlled by small doses of quinine. A latent infection developed. At the end of 5 months from the initial inoculation, the patient was superinfected with the same strain of parasite. This resulted in a mild attack of fever with reappearance of parasites and quick recovery without any treatment. A similar superinfection with the same strain a few weeks later caused an even milder attack. Other superinfections with this strain at the 7th and 8th months failed to produce any detectable clinical or parasitic results. They concluded that the patient was now immune to this strain. However, a superinfection at the end of 11 months with a Sardinian strain of *P. falciparum* caused a very severe attack of malaria. They state that their 'records contain other examples of the same kind'.

Watson (1932) considers that while Malays may have acquired a considerable immunity to the local strains of parasite in their own areas, yet the virulence of these strains may be raised by passage through mosquitoes and Indian emigrants. Under such conditions the immunity of the local inhabitants is no longer able to counteract the clinical effects of reinfection with this changed strain of parasite. Djaparidse (1932) thinks on epidemiological grounds that immunity to superinfection with *P. falciparum* is quickly lost. He believes

that this is accounted for by the acute and short duration which characterizes infection with this parasite, in contrast with the more chronic and prolonged nature of benign tertian infections, in which immunity lasts longer.

The epidemiological and other observations of many workers, more especially those of Daniels, Schüffner and Christophers, show that a tolerance to the effects of malarial infections develops among the older children and the adults in areas where malaria is hyperendemic. It appears to us that the time taken to produce this high degree of tolerance in any area, may depend upon the rapidity with which any individual in the area becomes infected and reinfected, *not only* with the same strain of parasite, but rather with the various strains and species commonly prevalent in that locality. When this has occurred and the individual has recovered spontaneously from the effects of the infections with each strain, a high degree of immunity will be produced. This tolerance may not be sufficient, however, to counteract the effects of infection with a foreign strain, against which the patient has not previously had any chance of developing an immunity.

### (3) CROSS-IMMUNITY IN HUMAN MALARIA

The fact that mixed infections are by no means rare in human malarial infections, indicates that, if any cross-immunity exist between different species of human Plasmodia, it cannot be of a very complete character. The findings in the peripheral blood, in such mixed infections, usually show that one species of parasite may predominate at certain times to the complete, or almost complete, exclusion of the other. At later examinations, however, the other parasite may show a predominance. This suggests that one species may have some adverse influence upon the development of the other. It may also be that conditions in the host suitable for the relapse of one species may differ from those favourable for the other species. Whether this is due to some common factor, such as a stimulation of the reticulo-endothelial system, or some more complex immunological mechanism, is uncertain, but is probably due to the latter.

Plehn (1926) reports that infection with *P. vivax* produced no tolerance to *P. falciparum*. He thinks, on the other hand, that a latent infection with *P. falciparum* may be reactivated by inoculation with *P. vivax*, but the former infection seemed to protect in some measure against the latter.

Ciuca, Ballif and Viéru (1928) found that an acquired tolerance to *P. vivax* caused a certain amount of resistance against superinfection with *P. malariae*, but not against *P. falciparum*. James (1931) also states that immunity to reinfection with *P. vivax* confers no protection against infection by *P. malariae* or by *P. falciparum*.

These results appear to indicate that, at least with the strains used by these workers, little or no cross-immunity is developed between the three common malarial parasites of man. It is interesting to compare these observations

with the results recorded by Gingrich (1932) with different species of bird *Plasmodia* (*vide supra*). The latter worker found that some species were capable of causing an effective cross-immunity against superinfection with some of the other species, while others appeared to have little or no cross-immunizing action.

#### SUMMARY.

A number of observations have been made on the effects of superinfection with different strains of the same species of human *Plasmodium*, and also on the cross-immunity produced to superinfection with a heterologous species. The results so far obtained have been fairly consistent but, as in the case of avian malaria, the number of experiments made and the variety of strains used have in most instances been comparatively few. Further work is required on these very important subjects.

The results so far recorded may be summarized as follows :

1. There is a large amount of evidence to support the view that there exist strains of the same species of human *Plasmodium*, which vary very considerably in their relative virulence to the human host, in their reaction to therapeutic agents, and in their power to infect Anophelines.

2. The information available suggests that the original virulence of a strain of *P. vivax* may be altered by frequent passage, through either the insect or animal hosts.

3. Chronic or latent infections with *P. vivax*, which have been allowed to run their natural course, confer a complete, or almost complete, immunity to superinfection with the same strain, either by mosquito bite or by blood inoculation. Little or no immunity is conferred, however, against a heterologous strain.

4. Chronic or latent infections with *P. falciparum* confer an immunity against superinfection with the same strain of parasite, but not against a heterologous strain.

5. The degree of immunity developed to superinfection with the same strain of either *P. vivax* or *P. falciparum*, appears to increase with the number of superinfections which the host has received with this strain.

6. A certain degree of tolerance to superinfection with the same strain of either *P. vivax* or *P. falciparum*, appears to be comparatively quickly acquired by the human host. This immunity appears to diminish rapidly when the parasites are completely eliminated from the body, either by natural or therapeutic means.

7. The degree of immunity produced by a natural cure, either clinical or permanent, appears to be much greater than when the cure is produced by therapeutic means.

8. A chronic or latent infection with either *P. vivax*, *P. falciparum*, or *P. malariae*, seems to confer little or no cross-immunity against superinfection with a different species of *Plasmodium*.

### PREVIOUS WORK ON MONKEY MALARIA.

Flu (1908), in working with *P. inui* var. *cynomolgi*, reported that previous infections with this parasite made the effects of subsequent inoculations with the same strain of parasite mild in character. Leger and Bouilliez (1913) tried to superinfect a specimen of *S. sinicus* with *P. inui*. This animal had a latent infection with the same strain of parasite, and attempts to superinfect it about 17th and 30th weeks, respectively, after the primary inoculation, failed to produce any detectable results. Blanchard and Langeron (1912), using *P. inui* var. *cynomolgi*, and Seidelin and Connal (1914) with *P. kochi* var. *macfiei*, have pointed out that the pathogenic effects of experimental infections may depend upon whether the animal inoculated has previously developed some immunity, as the result of previous infection. This view has been discussed by Sinton and Mulligan (1933).

Taliaferro (1932) has completed a few studies on the cellular basis for immunity to superinfection in monkeys infected with the American *Plasmodium*, *P. brasilianum*, a quartan-like parasite. He reports a high degree of immunity to superinfection following the initial acute infection. He also states that 'our results indicate clearly, however, that in sharp contrast to the sluggish phagocytosis of parasites by the macrophages of the normal animal, phagocytosis is rapid and more quickly effective in a previously infected animal. Just as in the case of avian malaria (Cannon and Taliaferro, 1931) immunity to superinfection rests upon a greater number of macrophages and a much greater phagocytic activity of those present'. 'So far no intermediary antibody has been associated with this cellular activity, but there are reasons for supposing that an opsonizing antibody may be formed locally which is of too low a concentration in the peripheral blood to be demonstrated by the technique so far employed'.

### SUMMARY.

There is some evidence to suggest that a considerable degree of immunity may be developed to superinfection with the same strain of monkey *Plasmodium*.

### EXPERIMENTAL INVESTIGATION OF IMMUNITY TO SUPERINFECTION IN MONKEY MALARIA.

Although the *Plasmodia* of monkeys seem eminently suitable for the investigation of superinfection, and for a comparison of the results with human infections, yet very little work seems to have been published on this subject. In the present paper, experiments are recorded on the immunity or tolerance produced to superinfection by either a homologous or a heterologous strain of parasite. It is hoped at a later date to report the results of superinfection of the same animal with several heterologous strains. Such work takes a long time, for a sufficient period must be allowed to elapse between each infection, to give the infected host ample opportunity to develop a high degree of tolerance

to each strain used. It is also necessary to allow for the occurrence of a long incubation period in any one case.

#### METHODS USED IN THE EXPERIMENTS.

##### (1) Technique of transmission of infection.

The *primary infection* in all the monkeys used in these experiments, with one exception, was induced by the inoculation of blood from another monkey, known to be infected with the strain which it was desired to transmit. The dose of infective blood varied from 0.1 c.c. to 0.5 c.c., according as to whether the donor showed a slight or a heavy parasitic infection. The route of administration varied in different animals, but as a rule was intraperitoneal. Equally successful results were obtained by subcutaneous or intramuscular injections.

The *superinfection* was effected in every case, by the inoculation of 0.25 c.c. of infected blood intraperitoneally. This dosage and route of administration was adhered to, because it was felt that any tendency to protein shock following the injection, with possible resultant relapse, would be more or less the same in every case. Using a standard dosage of 0.25 c.c. of blood in citrate-saline solution, no evidence of such shock was noted in any monkey. Similar doses of blood from normal monkeys were found, by experiment, to produce no perceptible effect on the behaviour of the parasitic infection in two monkeys having low-grade infections with *P. knowlesi*. Injections of larger doses (2 c.c.) of human blood intravenously, produced definite protein shock in such animals, associated with a reappearance of parasites in several monkeys in which these had not been detectable for a considerable time. It has been our experience that a dose of 0.25 c.c. of infected blood, given intraperitoneally, is sufficient to cause infection in a healthy susceptible monkey, even when the number of parasites in the peripheral blood is very scanty.

##### (2) Technique of blood examination.

So far as was practicable, daily examinations of the blood of all monkeys under experiment were carried out. In some instances, however, where the infections were being observed for very long periods (upwards of one year), and where a large series of animals were under experiment for various purposes, this was not possible. Periodical blood examinations were made, however, at intervals of about a week in all cases, and at more frequent intervals (often daily) when required for any special reason.

The thick-smear method was always employed, in conjunction with the thin-film method, for all routine examinations. The former method has the great advantage of saving much valuable time and of facilitating the detection of very scanty infections. The thin film is often useful in confirming the occurrence of mixed infections detected in the thick smears.

### (3) Tests for latent infections.

It was not considered that the failure to find parasites in the peripheral blood by routine examinations, even by the thick-smear method, was sufficient evidence to exclude the presence of latent infections. It was found that intravenous injections of foreign protein (e.g., doses of 2 to 3 c.c. of human blood) into monkeys with latent malarial infections, were often followed by the re-appearance of small numbers of parasites in the peripheral blood. Advantage was taken of this finding to ascertain whether certain monkeys, which had shown no parasites in the peripheral blood for long periods, were still infected. Similarly monkeys, which had been imported from localities where monkey malaria is endemic (e.g., *S. irus* from Singapore), were tested in this way. There is no conclusive evidence, however, to prove that a negative result indicates that the monkey is completely free from infection. With imported monkeys, the blood after protein shock was also injected into a susceptible animal (*S. rhesus*), as a further test for absence of latent infection.

### (4) The control of acute attacks of malaria.

The majority of superinfection experiments, recorded here, were carried out with various strains of *P. knowlesi* in *S. rhesus* monkeys. The extreme virulence of all strains of this parasite, so far isolated by us from monkeys, has made it impossible to obtain established chronic infections, without the use of drugs to control the initial attack (*vide infra*). With one exception in about 120 infections, it has been found that death resulted in every case where treatment of the initial attack was withheld. Generally speaking death also resulted, in the majority of cases in which treatment was not given during the very early stages of the acute attack. In some cases recovery was observed when treatment was started on the 3rd or 4th day of the attack, but such instances were the exception rather than the rule.

Quinine sulphate in doses of 2 to 3 grains daily was found to be sufficient to arrest the acute attack in most cases, provided treatment was started early. The drug was given in solution by the mouth and continued for from 2 to 5 days. Good results were also obtained by the early use of daily intramuscular injections of 0.0025 grm. plasmoquine, continued for 2 to 3 days. Quinine combined with plasmoquine, or the drugs given on alternate days in the doses mentioned, was attended with good results. Tebetren was tried in some cases but was not found to be any more effective than quinine, if indeed so good. Stovarsol proved very beneficial in the severe anæmias, so frequently observed after the acute attack.

Relapses were very common after the cessation of treatment. While some of these recovered spontaneously, many of them needed further treatment. The later relapses became progressively milder in character, and, where possible, the animal was allowed to overcome the relapse without therapeutic intervention.



## ORIGIN OF STRAINS OF MONKEY PLASMODIA.

Two species of monkey Plasmodia were used in the experiments about to be described, namely :

- (a) *Plasmodium knowlesi* Sinton and Mulligan, 1932.\*
- (b) *P. inui* var. *cynomolgi* Mayer, 1907.\*

**(a) Strains of *P. knowlesi*.**

Four strains of *P. knowlesi* were isolated from four different specimens of *S. irus* bought in Calcutta. These animals were said to have been imported from Singapore. The local brown monkey (*S. rhesus*) proved so susceptible to infection by blood inoculation with this parasite, that usually no difficulty was experienced in isolating apparently pure infections of each strain by sub-inoculation into this species of monkey.\* For convenience of description these four strains have been referred to as 'K<sub>1</sub>' strain, 'K<sub>2</sub>' strain, 'K<sub>3</sub>' strain and 'K<sub>4</sub>' strain. After inoculation into healthy *S. rhesus* monkeys, each of these strains was found to produce extremely severe infections. If untreated, these infections were associated with pernicious symptoms, and hæmoglobinuria was often seen. With one exception, death has resulted in every untreated infection.† The virulence of all four strains appears to be very similar.‡ No definite evidence has been obtained to indicate that the virulence of any of the strains has altered appreciably, as the result of repeated passage through specimens of *S. rhesus*.‡

In addition to the strains mentioned above, we have also used a strain of *P. knowlesi*, which was very kindly given us by Lieut.-Colonel R. Knowles, I.M.S., of the School of Tropical Medicine, Calcutta. The origin of the latter strain ('C' strain) was similar to that of the four strains isolated by us. No appreciable difference has been detected between the virulence of this strain and the other four strains of *P. knowlesi* used in our work.

**(b) Strains of *P. inui* var. *cynomolgi*.**

A single strain of this species ('Cyn' strain) has been used. This was also obtained from a specimen of *S. irus* bought in Calcutta, and said to have come from Singapore. When originally examined this monkey was found to

\* *Vide* Sinton and Mulligan (1933).

† In a series of over 120 inoculation infections with *P. knowlesi* in *S. rhesus*, only one monkey has recovered spontaneously from the initial acute attack. Death occurred in every untreated case, and also in a number in which treatment was not commenced early.

‡ The extreme virulence of all these strains makes it very difficult to detect any variations in their virulence to *S. rhesus*, because the infections, if untreated, almost invariably lead to death with all the strains. Similarly, alterations in virulence as the result of animal passage are difficult or impossible to estimate.

have a mixed infection of both *P. knowlesi* and *P. inui* var. *cynomolgi*.\* The latter species of *Plasmodium* was isolated in pure form after passage to *S. rhesus*, and remained pure on subsequent passage to other monkeys of the same species, both after blood inoculation and after infection by mosquito bites.\*

FEATURES OF MALARIAL INFECTIONS PRODUCED BY *P. KNOWLESI* AND  
*P. INUI* VAR. *CYNOMOLGI*.

The features of these infections have been described more fully by Sinton and Mulligan (1933), but a short summary here should facilitate a study of the results of the experiments recorded below. The severity of the infections varies considerably in the different species of *Silenus* used in these experiments.

(1) Malarial infections due to *P. knowlesi*.

(a) In *Silenus irus*. This is the natural host of this *Plasmodium*. In such animals no symptoms were observed and the infections were only detectable by blood examination, at least in all the specimens examined by us. In inoculation infections, no clinical signs or symptoms were observed, except for a daily rise of temperature in the early stages. Parasites are found in appreciable numbers in these early stages, but the numbers soon decrease. Subsequently they are very scanty, or undetectable, for long periods in the peripheral blood.

(b) In *Silenus rhesus*. When the infection is transmitted by blood inoculation to healthy monkeys of this species, a very severe attack develops rapidly. During this acute attack the animals show high fever, great malaise and acute pernicious symptoms, often with hæmoglobinuria. If adequate treatment be commenced early in the acute attack, the progress of the disease is arrested, and a chronic infection follows. As a rule such chronic infections are preceded or interrupted by one or more severe relapses†, which may require treatment or may undergo spontaneous recovery. Subsequently a low-grade chronic infection is developed which may persist for months, or even more than a year, its presence being recognized by the occasional occurrence of scanty parasites in the peripheral blood. Death is the rule in cases which receive no treatment, or where the commencement of treatment is too long delayed.

(c) In *Silenus sinicus*. Only two monkeys of this species were infected, and these were found to develop an infection similar to that seen in *S. rhesus*, but the acute attack appeared to be less severe. Knowles and Das Gupta (1932), working with 'C' strain of *P. knowlesi*, found that in four specimens

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\* Vide Sinton and Mulligan (1933).

† In this work the term 'relapse' has been used to denote an increase or recrudescence of parasites in such numbers as to cause a distinct disturbance in the health of the animal. This may be shown by fever or some obvious signs of illness or malaise. The periodical reappearance of parasites in scanty numbers, without any apparent effect on the health of the animal, is not considered to be a relapse in the sense employed in these experiments. Such reappearance can often be detected only by daily blood examinations and, in the absence of these examinations, such increases are likely to be missed.

of *S. sinicus* (*M. radiatus*) the resultant infections were milder than in *S. rhesus*, and that recovery was usually spontaneous.

**(2) Malarial infections due to *P. inui* var. *cynomolgi*.**

(a) In *Silenus irus*. No clinical symptoms or signs of the infection were observed. The infection was detectable only by blood examination. As all the infected animals observed by us were suffering from chronic malaria due to this parasite, we have been unable to determine whether any symptoms occur in freshly infected normal animals of this species.

(b) In *Silenus rhesus*. After inoculation into healthy specimens of this monkey, moderately heavy parasitic infections developed. In spite of this the animals appeared to suffer little or no inconvenience. The parasites appeared after an incubation period of about 5 days and increased gradually until about the 10th day, when the infection subsided spontaneously. A chronic low-grade infection may persist for months, its presence being recognized by the periodical appearance of scanty parasites in the peripheral blood.

(c) In *Silenus sinicus*. The course of the infection in this species appears to be similar to that described in *S. rhesus*.

The clinical effects of infection with *P. inui* var. *cynomolgi* in both *S. rhesus* and *S. sinicus*, were markedly milder than those produced by *P. knowlesi* in the same species.

**RESULTS OF ATTEMPTS TO SUPERINFECT MONKEYS WITH HOMOLOGOUS STRAINS OF MONKEY PLASMODIA.**

Seven experiments have so far been completed, to determine whether chronic or latent infections with certain strains of monkey Plasmodia would confer any immunity or tolerance to superinfection with the same strains. In the case of *P. knowlesi*, the monkeys selected for superinfection were all suffering from established infections of considerable duration (64 to 343 days) at the time of superinfection. All these animals were apparently in excellent health. In every instance except one, these monkeys had suffered from an initial acute attack of malaria, the course of which had been arrested by appropriate treatment (quinine, plasmoquine, tebetren or a combination of these drugs). In all cases the acute attack had been followed by one or more relapses. These relapses varied in number and intensity in different animals. Usually the first relapse was a severe one and not infrequently treatment was required to save the life of the animal. Subsequent relapses tended to become progressively milder, and recovery was often spontaneous, although in some cases treatment was required on these occasions. The infection in all cases eventually became chronic or latent. At this time parasites were either absent, or present only in small numbers in the peripheral blood over long periods. At the time of superinfection, parasites, if present, were to be seen only in scanty numbers. No parasites were observed for some months in a few instances, but, because examinations were often made only at intervals of about a week in

some of such animals, it is possible that the occasional presence of parasites in the peripheral blood may have been missed.

Only one animal infected with *P. inui* var. *cynomolgi* was superinfected with the same strain of parasite.

A brief summary of the history of each monkey used in these experiments, and the results of superinfection with a homologous strain of parasite are given below. The seven experiments completed have been denoted by the letters 'a' to 'g' for ease of reference.

### A. Superinfections with homologous strains of *P. knowlesi*.

#### (1) 'C' strain.

##### (a) Monkey No. 7 (*S. rhesus*).

*History of primary infection.* Infection produced by direct blood inoculation\*; parasites appeared on 3rd day†; severe attack developed, rapidly reaching a maximum on 7th day, when animal showed pernicious symptoms; parasites very numerous on 6th to 11th days; quinine treatment‡ given on 6th, 7th and 8th days; acute iclapse occurred on 20th day, but recovery was spontaneous; subsequently infection became chronic with scanty parasites on and off until 341st day, during which period the monkey appeared to be in excellent health.

*History of superinfection.* Superinfected 343rd day with same strain. Scanty parasites observed on 350th, 351st, 353rd and 354th days; blood examined on 11 occasions up to 379th day, but parasites found on only one occasion (371st day); no clinical symptoms noted after superinfection.

*Result of superinfection.*—No change in the course of the existing infection could be detected.

##### (b) Monkey No. 29 (*S. rhesus*).

*History of primary infection.* Parasites detected on 4th day and increased rapidly to reach maximum on 6th day; severe attack with pernicious symptoms; quinine treatment given on 6th and 7th days arrested attack; severe relapse occurred on 11th day, but recovery followed quinine administration on 12th day; no further relapses of severe nature occurred, and animal acquired a chronic infection with periodical appearance of scanty parasites until 152nd day; subsequently no parasites were observed (irregular examinations) up to 257th day.

*History of superinfection.* Superinfected 258th day with same strain. Parasites reappeared on 260th day and were observed daily in scanty numbers until 269th day; parasites were relatively more abundant on 265th day; examinations made almost daily up to 301st day revealed parasites on only two subsequent occasions.

*Result of superinfection.*—No acute attack developed, but a very slight transient increase in the number of parasites in the peripheral blood was noted.

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\* All the infections and superinfections recorded in these summaries were produced by direct blood inoculation, unless otherwise stated.

† In recording these results the day of the disease has been reckoned from the date on which the animal received its primary infective inoculation.

‡ Fuller details of treatment have been given above.

(c) Monkey No. 529 (*S. rhesus*).

*History of primary attack.* Parasites detected on 4th day; acute attack developed, becoming maximal on 10th day; recovery following treatment (quinine and plasmoquine) on 7th, 8th and 9th days; no severe relapse occurred shortly after acute attack, but infection remained scanty until 65th day, after which no parasites observed until 175th day; a severe relapse occurred reaching maximum on 178th day; recovery followed quinine treatment on 177th, 178th and 179th days; subsequently parasites were scanty or absent until 263rd day, and thereafter absent (daily examinations) until 311th day.

*History of superinfection.* Superinfected on 312th day with same strain. No parasites detected in peripheral blood up to 369th day (daily examinations).

*Result of superinfection.*—No return of clinical symptoms, nor reappearance of parasites in the peripheral blood, was detected.

(2) 'K<sub>1</sub>' strain.(d) Monkey No. 66 (*S. rhesus*).

*History of primary infection.* Parasites detected on 5th day and increased rapidly to reach maximum on 8th day; severe attack with pernicious symptoms; quinine treatment given daily from 5th to 9th days, and from 11th to 13th days, arrested attack; relapse occurred on 19th day and again on 23rd day, but recovery followed a single dose of quinine on each occasion; further relapses requiring quinine treatment occurred on 26th, 41st, 48th and 67th days. Eventually infection became chronic and relapses ceased; parasites observed on and off up to 200th day.

*History of superinfection.* Superinfected on 209th day with same strain. No marked change observed in parasite findings after superinfection, although daily examinations were made up to 251st day; slight transient increase in the number of parasites noted on 220th day.

*Result of superinfection.*—No appreciable change was detected.

(3) 'K<sub>2</sub>' strain.(e) Monkey No. 64 (*S. rhesus*).

*History of primary infection.* Parasites detected on 8th day, but disappeared after quinine treatment given on same day; parasites reappeared on 14th day and increased rapidly, reaching maximum on 18th day; very acute attack which recovered with quinine treatment given on 16th, 17th and 19th days; two relapses requiring quinine treatment occurred on 24th day (2 days' treatment) and on 79th day (3 days' treatment); subsequently mild relapses occurred with spontaneous recovery; infection gradually became chronic with periodical appearance of scanty parasites up to 211th day.

*History of superinfection.* Superinfected on 212th day with same strain. Beyond a slight transient increase in the number of parasites on 233rd, 235th and 239th days, parasites remained scanty or absent at daily examinations up to 250th day.

*Result of superinfection.*—No appreciable change in the infection was detected.

(4) 'K<sub>4</sub>' strain.(f) Monkey No. 110 (*S. rhesus*).

*History of primary infection.* Parasites detected on 5th day, and increased rapidly to become maximal on 7th and 8th days; severe symptoms; infection controlled by treatment (quinine and plasmoquine) on 6th and 7th days; relapse occurred on 12th day, but was arrested by treatment (quinine and plasmoquine) on 12th, 13th and 14th days; a second relapse on 21st day recovered spontaneously; subsequent relapses of a progressively mild nature occurred, but required no treatment; infection eventually became chronic with scanty parasites present daily up to 63rd day.

*History of superinfection.* Reinfectd on 64th day with same strain. Beyond slight transient increase in the number of parasites on 81st day, parasites were scanty or absent at daily examinations up to 113th day.

*Result of superinfection.*—No appreciable change was produced by superinfection with a homologous strain.

**B. Superinfection with a homologous strain of *P. inui* var. *cynomolgi*.**(1) 'Cyn<sub>1</sub>' strain.(g) Monkey No. 139 (*S. rhesus*).

*History of primary infection.* Infection induced by bites of infected *A. annularis* (*A. fuliginosus*); parasites detected on 14th day after first bites; parasitic attack of moderate severity developed gradually, reaching maximum between 19th and 22nd days; no perceptible clinical symptoms; spontaneous recovery followed, and blood became free from parasites on 27th day.

*History of superinfection.* Superinfected on 27th day by blood inoculation with same strain. Scanty parasites detected at daily examinations from 29th to 40th days; no symptoms apparent.

*Result of superinfection.*—A very mild parasitic infection without clinical symptoms developed. It is doubtful whether the parasites seen were the result of superinfection or of a reappearance of the parasites of the original infection. The time allowed for the development of immunity and the period of observation after superinfection were, however, so short that no definite conclusions seem justifiable.

**Summary of attempts to superinfect monkeys with homologous strains of Plasmodia.**

Under the conditions of our experiments, the results recorded above\* appear to indicate either that (1) superinfection with a homologous strain of either *P. inui* var. *cynomolgi* or *P. knowlesi* fails to produce a reinfection in *S. rhesus*, or that (2) if a reinfection be produced, its effects are so mild that it can only be recognized by a very slight transient increase in the number of parasites in the peripheral blood.

In four of the six monkeys superinfected with *P. knowlesi* [*vide* experiments (a), (c), (e) and (f)], no changes in the peripheral-blood findings, which could be attributed to reinfection, were noticeable. In the remaining two monkeys

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\* *Vide* Table A also.

[experiments (b) and (d)], the very slight and transient increase in the number of parasites may possibly have been connected with reinfection. It was, however, impossible to exclude a reappearance or slight increase of the primary infection.

In the single experiment with *P. inui* var. *cynomolgi* [experiment (g)], it is impossible to say whether a superinfection with the same strain was in any way responsible for the presence of the scanty parasites in the peripheral blood during the subsequent observation period. This experiment suggests, however, that superinfection with the same strain of *P. inui* var. *cynomolgi*, by direct-blood inoculation into a monkey suffering from a mosquito-borne infection, is probably not associated with a fresh attack of any significance.

#### RESULTS OF ATTEMPTS TO SUPERINFECT MONKEYS WITH HETEROLOGOUS STRAINS OF MONKEY PLASMODIA.

As attempts to produce acute infections of malaria by superinfecting monkeys with homologous strains of monkey Plasmodia had failed, it was decided to investigate the effects of superinfection with heterologous strains. In order to make the results comparable, monkeys were selected for these experiments which corresponded as nearly as possible to those used in the previous work. The dosage of infected blood and the route of administration was the same in both series. Thirteen monkeys with chronic low-grade or latent infections were chosen. All these animals appeared to be in excellent health at the time of superinfection. None showed any signs of disease beyond the periodical occurrence of parasites in scanty numbers in the peripheral blood.

The results of these experiments are summarized below and, as before, a brief account of the course of the primary infection and of the superinfection is given. *P. knowlesi* was the parasite used, and the origin of the different strains has already been recorded. The 13 experiments completed in this series have been denoted as 'h' to 't', for ease of reference.

#### (A) Superinfections on primary infections of *P. knowlesi*, strain 'C'.

(1) Superinfection of *P. knowlesi*, strain 'C' with strain 'K<sub>1</sub>'.

(h) Monkey No. 9 (*S. rhesus*).

*History of primary infection.* Parasites detected on 5th day and increased rapidly; acute attack of extreme severity, becoming maximal on 9th day when animal showed severe pernicious symptoms, including hæmoglobinuria\*; quinine treatment given on 8th, 9th and 10th days, after which recovery occurred; severe relapse on 19th day, stopped by a single dose of quinine; subsequently two relapses occurred on 131st and 180th days, but both recovered spontaneously; the infection then became chronic with periodical appearance of parasites until 310th day; later no parasites observed (irregular examinations) up to time of superinfection.

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\* This was the only animal in our experiments which recovered after the occurrence of hæmoglobinuria.

*History of superinfection.* Superinfected on 343rd day with K<sub>1</sub> strain; parasites detected on 347th day and increased rapidly; acute attack of moderate severity becoming maximal on 350th day; parasites decreased in numbers on 352nd and 353rd days but increased again on 354th, subsequently decreasing to scanty numbers without treatment; parasites present in blood in appreciable numbers (daily examinations) up to 378th day, but no relapse observed.

*Result of superinfection.*—A moderately acute attack with spontaneous recovery occurred. No relapse was observed during observation up to 35 days after reinfection.

(i) Monkey No. 530 (*S. rhesus*).

*History of primary infection.* Parasites detected on 5th day; severe clinical and parasitic attack necessitating quinine on 7th, 8th and 9th days; no very severe relapse occurred, but some of moderate severity on 13th, 21st and 36th days, followed by spontaneous recovery in each case; parasites continued in peripheral blood until 133rd day, but subsequently none found up to 226th day.

*History of superinfection.* Superinfected on 226th day with K<sub>1</sub> strain; parasites detected on 233rd day and increased rapidly, causing very severe clinical attack with pernicious symptoms; plasmoquine treatment commenced on 235th day, but animal developed hæmoglobinuria and died same day.

*Result of superinfection.*—An extremely severe attack with pernicious symptoms and hæmoglobinuria developed, followed by death.

(j) Monkey No. 7. (*S. rhesus*).

*History of primary attack.* Very severe initial attack treated with quinine; later an acute relapse with spontaneous recovery; animal was then found refractory to superinfection with a homologous strain on 343rd day [*vide* experiment (a)].

*History of superinfection.* Superinfected on 379th day with K<sub>1</sub> strain; parasites detected on 384th day; acute attack of great severity developed, becoming maximal on 388th day; recovery followed treatment (plasmoquine and quinine) on 388th, 389th and 390th days; no relapse occurred within an observation period (daily blood examinations) extending to 426th day, but parasites seen in scanty numbers on many occasions.

*Result of superinfection.*—In spite of a previous superinfection with a homologous strain, a very severe attack developed. This was controlled by treatment and no subsequent relapse was observed up to 47 days after reinfection.

(k) Monkey No. 29. (*S. rhesus*).

*History of primary infection.* Very severe attack controlled by treatment; one severe relapse also requiring treatment; superinfection with same strain of parasite on 258th day produced slight transient increase in parasites [*vide* experiment (b)].

*History of superinfection.* Superinfected on 309th day with K<sub>1</sub> strain; parasites detected on 314th day and very acute attack developed quickly, becoming maximal on 317th day; plasmoquine treatment was given on 317th and 318th days, and the animal recovered; no relapse occurred during an observation period extending to 356th day (daily blood examinations), but scanty parasites found on all but 2 days.



*Result of superinfection.*—A very severe attack developed with very high parasite counts and serious clinical symptoms. Recovery followed plasmoquine treatment and no relapse was observed during 47 days after superinfection.

(2) *Superinfection of P. knowlesi, strain 'C' with strain 'K<sub>1</sub>'.*

(l) Monkey No. 39 (*S. rhesus*).

*History of primary infection.* Parasites detected on 8th day, and increased rapidly to reach maximum on 11th day; resultant attack very severe with pernicious symptoms; recovery followed febetren treatment given daily from 9th to 19th days; no severe relapse observed; a low-grade infection developed, during which parasites in scanty numbers seen from time to time up to 195th day.

*History of superinfection.* Superinfected on 203rd day with K<sub>1</sub> strain; parasites detected on 216th day and became maximal on 222nd day; acute attack with marked symptoms developed and was controlled by quinine and stovarsol given on 222nd, 223rd and 224th days; no subsequent relapses observed, but parasites usually present in scanty numbers (23 examinations) up to 286th day.

*Result of superinfection.*—The superinfection was followed by an acute attack of considerable severity, which was controlled by treatment. No relapse was observed up to 83 days after superinfection.

(m) Monkey No. 25 (*S. rhesus*).

*History of primary infection.* Parasites detected on 6th day, and increased rapidly until 9th day, when a severe attack with pernicious symptoms had developed; plasmoquine treatment on 9th and 10th days was followed by recovery; a severe relapse occurred on 20th day, requiring treatment (quinine) on 20th and 21st days; subsequently two moderately severe relapses developed at 103rd and 122nd days with spontaneous recovery; in intervals between relapses parasites were scanty or absent (daily examinations); after 125th day no parasites seen, although animal observed (irregular blood examinations) until 260th day.

*History of superinfection.* Superinfected on 260th day with K<sub>1</sub> strain; parasites detected on 266th day; an extremely severe attack with pernicious symptoms developed rapidly; hæmoglobinuria was seen on 271st day and death took place the same day; no treatment was given.

*Result of superinfection.*—A very severe attack occurred followed by hæmoglobinuria and death.

(3) *Superinfection of P. knowlesi, strain 'C' with strain 'K<sub>4</sub>'.*

(n) Monkey No. 21 (*S. rhesus*).

*History of primary infection.* Parasites detected on 8th day and increased rapidly, becoming maximal on 11th day; acute attack with severe symptoms arrested by plasmoquine treatment on 12th and 13th days; blood remained negative from 15th to 18th days; severe relapse occurred on 24th day, but recovery followed a single dose of plasmoquine; no subsequent relapse, and infection became a low-grade chronic one, with scanty parasites on and off till 133rd day; daily blood examinations till 294th day revealed parasites on only one occasion (127th day); protein shock on 294th day (2 c.c. human blood intravenously) caused reappearance of parasites on the same evening and subsequently daily till 309th day; parasites also present on 305th and 306th days, but not seen again up to 357th day (35 examinations).

**History of superinfection.** Superinfected on 358th day with  $K_1$  strain; parasites detected on 363rd day, but no acute attack developed; a mild parasitic infection without clinical symptoms was observed up to 408th day; on 409th day a very acute attack with pernicious symptoms developed; treatment (quinine, plasmoquine and stovarsol) was given on 409th and 410th, but death occurred on 411th day.

**Result of superinfection.**—A mild parasitic infection was observed, but no acute attack developed within 50 days of superinfection. At the end of this time an extremely severe attack with pernicious symptoms occurred, and vigorous treatment was required, but in spite of this the animal died. It seems almost certain that this severe attack was the result of superinfection with the heterologous strain ( $K_1$ ). It is highly improbable that the acute attack was a relapse of the primary infection. No clinical relapse had been observed in this monkey for almost a year prior to superinfection, in spite of the production of a severe protein shock on the 294th day. The cause of the delay in the occurrence of the acute attack following superinfection is uncertain. It may be that either the very prolonged and chronic course of the primary infection, or the administration of a severe protein shock, was responsible for a somewhat greater degree of immunity to superinfection than was observed in most other monkeys. A long incubation period of an apparently similar nature was observed in Monkey No. 74 [*vide* experiment (o)].

**(B) Superinfections on primary infections with *P. knowlesi*, strain ' $K_1$ '.**

(1) *Superinfection of *P. knowlesi*, strain ' $K_1$ ' with strain 'C'.*

(o) Monkey No. 74 (*S. rhesus*).

**History of primary infection.** Parasites detected on 3rd day and increased very rapidly; very acute attack with severe pernicious symptoms, becoming maximal on 7th day; quinine and plasmoquine treatment given from 4th to 10th days and animal recovered; acute relapse on 21st day necessitated quinine treatment on 21st, 23rd and 24th days; two subsequent relapses occurred (42nd and 61st days) requiring treatment; infection then became chronic and parasites were found periodically until 168th day.

**History of superinfection** Superinfected on 179th day with strain C; parasites detected in scanty numbers from 185th to 188th days, but were absent at subsequent examinations made daily up to 215th day; as the result of an oversight this monkey was again superinfected with the primary ( $K_1$ ) strain on 216th day; parasites were found on 222nd day and an acute pernicious attack developed rapidly, resulting in death on 225th day.

**Result of superinfection.**—No acute attack developed within 42 days of superinfection with the heterologous (C) strain but, after the expiry of this period, an acute pernicious attack occurred and resulted in the death of the monkey. As this animal had been superinfected with the primary ( $K_1$ ) strain in the interval between superinfection with the heterologous (C) strain and the occurrence of death, it is not possible to determine definitely whether death was caused by the homologous or the heterologous strain. In a previous experiment [*vide* experiment (d)] an established infection with  $K_1$  strain was found

to be associated with a complete immunity to superinfection with the same strain. Similarly established infections with all other strains, so far investigated, have been found to be associated with complete or almost complete immunity to superinfection with homologous strains. In the light of this evidence it is very difficult to believe that such an acute, and rapidly fatal, infection could be attributable to superinfection with the homologous strain. On the other hand it seems highly probable that the heterologous strain (C) was responsible for the production of this extremely virulent attack and that, as in the case of Monkey No. 21 [*vide* experiment (n)], the incubation period was prolonged beyond the usual time.

**(C) Superinfections on primary infections with *P. knowlesi*, strain 'K<sub>2</sub>'.**

(1) *Superinfection of P. knowlesi, strain 'K<sub>2</sub>' with strain 'C'.*

(p) Monkey No. 531. (*S. rhesus*).

*History of primary infection.* Parasites detected on 30th day\*; no acute attack developed and parasites were present in only scanty numbers up to 110th day †, when an acute exacerbation occurred; no treatment was given and the acute phase underwent spontaneous recovery; scanty parasites observed on daily examinations up to 127th day.

*History of superinfection.* Superinfected on 127th day with strain C; scanty parasites present at time of inoculation, but a definite increase was observed on 133rd day; a typical acute attack with marked constitutional symptoms developed quickly, and the animal was moribund on 138th day; no treatment was given. (This

\* This monkey was first inoculated in an attempt to establish this strain from the original host (*S. irus*). As no parasites appeared after 12 days (a longer incubation period than any previously seen by us at that time) the monkey was again inoculated, as it was thought that there might have been some error in technique. When no parasitic infection could be detected during a further observation period of 8 days, the monkey was inoculated for the third time, as it seemed as if this animal might possibly have a natural immunity. Parasites appeared 30 days after this third inoculation, but it is impossible to say which of the three inoculations was responsible for the infection which eventually resulted.

In recording the results of experiments carried out with this monkey, the day on which parasites appeared has been reckoned from the date of the third inoculation.

In the case of another monkey [*vide* experiment (q)] inoculated with the same strain and at the same time, no parasites could be detected within 20 days of first inoculation. A second inoculation was then given and parasites appeared 10 days later.

This strain (K<sub>2</sub>) was the only one with which any difficulty was experienced in establishing in *S. rhesus* from the original host. A possible explanation is that both these monkeys were much older than the monkeys usually employed in our experiments. In no other case, however, were such prolonged incubation periods observed in primary infections, even when large monkeys were used.

† This monkey was the only one in a very large series inoculated with *P. knowlesi*, in which no acute attack developed, and which survived the primary infection without the intervention of therapeutic measures.

animal was chloroformed, when moribund, to obtain parasites in bulk for other experiments.)

**Result of superinfection.**—A very severe attack occurred within 6 days of superinfection, which rendered the animal moribund.

(q) Monkey No. 533 (*S. rhesus*).

**History of primary infection.** Parasites detected on 11th day; a very acute attack developed quickly becoming maximal on 14th day; recovery followed treatment (quinine and plasmoquine) on 14th, 15th and 16th days; no severe relapse occurred, but slight fluctuations in the number of scanty parasites noted during period of chronic infection up to 116th day.

**History of superinfection.** Superinfected with 'C' strain on 117th day; parasites present in scanty numbers at time of superinfection; definite increase in number of parasites on 123rd day, and an acute pernicious attack developed quickly; no treatment given; animal died on 130th day.

**Result of superinfection.**—A very severe attack developed, which terminated fatally in the absence of treatment.

(2) *Superinfection of P. knowlesi, strain 'K<sub>2</sub>' with strain 'K<sub>1</sub>'.*

(r) Monkey No. 82 (*S. sinicus*).

**History of primary infection.** Parasites detected on 5th day; very numerous parasites associated with severe constitutional symptoms observed on 8th day; treatment (quinine and plasmoquine) given on 8th and 9th days, arrested acute attack, and blood became free from parasites on 17th and 18th days; no relapse occurred and monkey remained healthy, although parasites found in scanty numbers until 119th day; none found thereafter (6 examinations) up to 172nd day.

**History of superinfection.** Superinfected on 173rd day with K<sub>1</sub> strain; parasites detected on 180th day and moderately heavy infection ensued, reaching maximum on 189th day; no treatment given; acute attack subsided spontaneously; parasites disappeared on 191st day; stovarsol given on 189th day, as monkey very anæmic; parasites seen occasionally in scanty numbers up to 204th day.

**Result of superinfection.**—An acute infection developed with spontaneous recovery.

#### (D) **Superinfections on primary infections of *P. knowlesi*, strain 'K<sub>3</sub>'.**

(1) *Superinfection of P. knowlesi, strain 'K<sub>3</sub>' with strain 'K<sub>1</sub>'.*

(s) Monkey No. 80 (*S. rhesus*).

**History of primary infection.** (i) Primary attack:—Parasites detected on 8th day; quinine treatment given on 9th and 10th days controlled development of attack temporarily; subsequent severe attack developed (14th to 18th days), but recovery followed treatment (quinine and plasmoquine) given on 14th, 17th, 19th and 22nd days; relapse occurred on 31st day, but recovery spontaneous; no further relapse occurred, but parasites seen periodically in scanty numbers till 124th day. (ii) Superinfection with *P. inui* var. *cynomolgi* (strain 'Cyn<sub>1</sub>') :—Superinfection on 126th day resulted in heavy parasitic infection with latter parasite; recovery spontaneous with subsequent low-grade infection with both species of parasite up to 206th day.

*History of superinfection.* Superinfected on 207th day with  $K_1$  strain; parasites (*P. inui* var. *cynomolgi*) present at time of superinfection; acute attack due to *P. knowlesi* developed on 224th day; quinine treatment required for 1 day; scanty parasites present up to 240th day.

*Result of superinfection.*—An acute attack, requiring treatment, developed after an incubation period of 17 days.

**(E) Superinfection on 'primary infection of *P. knowlesi*, strain ' $K_4$ '.**

(1) *Superinfection of *P. knowlesi*, strain ' $K_4$ ' with strain ' $K_1$ '.*

(t) Monkey No. 107 (*S. rhesus*).

*History of primary infection.* Parasites detected on 10th day and, in spite of treatment (quinine) from 11th to 19th days, an acute attack developed; attack at maximum on 13th and 14th days; severe parasitic relapse on 22nd day with spontaneous recovery; subsequently no further relapses; parasites present in scanty numbers till 93rd day (almost daily examinations).

*History of superinfection.* Superinfected on 93rd day with  $K_1$  strain; parasites present at time of superinfection and began to increase on 103rd day; very acute attack developed quickly and in absence of treatment the animal died on 107th day.

*Result of superinfection.*—A very acute attack developed, which, in the absence of treatment, proved fatal.

## DISCUSSION OF RESULTS OF EXPERIMENTS ON SUPER-INFECTION WITH HETEROLOGOUS STRAINS.

The thirteen experiments summarized above suggest that a chronic infection with one strain of *P. knowlesi* does not confer an effective immunity against the occurrence of an acute malarial attack following upon superinfection with a different strain of the same parasite.

It will be seen, however, in experiments (n), (o) and (s) that the acute attacks occurred only after an unusually prolonged incubation period. Whether this is a common occurrence with the strains involved, will require further investigation.

Although a chronic infection with one strain does not appear to protect against superinfection with a different strain, yet it is interesting to note the absence of relapses in the secondary infections. No relapse after the acute attack was noted in any of the 8 animals which survived superinfection. On the other hand, in only 2 out of 19 animals was no relapse recorded during the primary infection. This suggests that some degree of immunity or tolerance is produced by the primary infection, but that the virulence of these strains of *P. knowlesi* is so great that the effects of such immunity are, in most instances, difficult or impossible to detect during the acute attacks of the superinfection. Such an idea is supported by the fact that in 2 out of 13 experiments, the acute attack of the superinfection cleared up spontaneously. On the contrary, in only 1 out of about 120 cases was such an occurrence recorded during primary infections. The occurrence of 3 instances of unusually prolonged incubation

after superinfection in a series of 13 experiments is also suggestive, as compared with only 2 such occurrences among about 120 primary infections.

One explanation of these findings might be that some 'immunity', possibly of a 'species' character, is produced. Such immunity, while insufficient to control the virulence of the superinfecting strain to such an extent as to prevent an acute attack, or even death, is yet sufficient to prevent the occurrence of relapses of the clinical symptoms. Another possible interpretation is that the slight degree of immunity produced, may not even be of a 'species' character. It may be due rather to a general non-specific stimulation of the reticulo-endothelial system, such as occurs in any malarial infection (Mulligan, 1929, 1931; Gay, 1931; Cannon and Taliaferro, 1931; Taliaferro, 1932). On the other hand, the immunity to homologous strains of parasite appears to be of a much more specific nature, and to be of quite a different character to this general activation of the reticulo-endothelial system.

It must be remembered, however, that our 'strains' were originally separated, only because each had been isolated from a different monkey. All these original hosts came from the Federated Malay States, where monkey malaria is apparently a common disease in nature. Under such conditions it was reasonable to expect that any two animals might have been infected with the same strain, or that any one animal might have had a mixed infection with two or more strains. This was especially the case with strains  $K_1$ ,  $K_2$  and  $K_3$ , which came from animals received in the same consignment. Up to the present, however, we have been unable to obtain any definite evidence that the strains used by us were either mixed or closely allied. It is possible that some such conditions may be responsible, at least in part, for the differences noted in some instances between the courses of the primary and the secondary infections, *e.g.*, absence of relapses, occurrence of spontaneous recovery and prolongation of the incubation period. Further work will be needed before any definite conclusions can be drawn between the different parts played by 'species' immunity, non-specific reticulo-endothelial activity and mixed or allied strains.

#### **Summary of the results of superinfection of monkeys with heterologous strains of *P. knowlesi*.**

The results obtained, under the conditions of our experiments, have been given in Table A, and these may be summarized as follows:—

(a) Chronic infections with strain 'C' conferred little or no immunity against the occurrence of an acute malarial attack, produced by superinfection with strain ' $K_1$ ' (4 experiments), nor with strain ' $K_2$ ' (2 experiments), nor with strain ' $K_4$ ' (1 experiment). The incubation period in the last instance was, however, very prolonged.

(b) A chronic infection with strain ' $K_1$ ' was probably associated with no complete immunity against the occurrence of an acute malarial attack, produced by superinfection with strain 'C' (1 experiment). The incubation period was

probably prolonged, as in the case of superinfection of strain 'K<sub>4</sub>' on strain 'C'.

(c) Chronic infections with strain 'K<sub>2</sub>' conferred little or no immunity against the occurrence of an acute malarial attack, after superinfection with strain 'C' (2 experiments), nor against strain 'K<sub>1</sub>' (1 experiment with *S. sinicus*).\*

(d) A chronic infection with strain 'K<sub>3</sub>' was not associated with an immunity against the occurrence of an acute malarial attack produced by superinfection with strain 'K<sub>1</sub>' (1 experiment). The incubation period was, however, prolonged up to 17 days.

(e) A chronic infection with strain 'K<sub>4</sub>' conferred no immunity against the occurrence of an acute malarial attack after superinfection with strain 'K<sub>1</sub>' (1 experiment).

(f) A chronic or latent infection with one strain of *P. knowlesi* appears to confer some tolerance to the clinical effects of superinfection with a different strain. This is shown by a greater tendency for the acute attack of superinfection to recover spontaneously, and by the diminished tendency to clinical relapse after this attack.

#### RESULTS OF EXPERIMENTS ON CROSS-IMMUNITY BETWEEN *P. KNOWLESI* AND *P. INUI* VAR. *CYNOMOLGI*.

Three experiments were made to determine whether a chronic or latent infection with *P. knowlesi* produced any immunity against a superinfection with *P. inui* var. *cynomolgi* and *vice versa*.

##### (1) *P. inui* var. *cynomolgi* superinfected on *P. knowlesi*.

###### (u) Monkey No. 121. (*S. irus*).

*History of primary infection* (*P. knowlesi*, 'C' strain). This monkey was said to have been imported from Singapore; the possibility of a natural infection had to be considered, but no parasites were found on repeated blood examination, nor after protein shock produced by intravenous injection of 2 c.c. of human blood. Parasites detected on 8th day after inoculation with 'C' strain *P. knowlesi*; a very mild infection resulted, with no symptoms beyond slight daily rise of temperature in early stages; recovery spontaneous and no relapse occurred.

*History of superinfection*. Superinfected on 14th day with *P. inui* var. *cynomolgi* ('Cyn.' strain); parasites detected on 23rd day; no acute attack developed; parasites of both species seen in scanty numbers on and off up to 64th day; on some occasions marked predominance of one or other species.

*Result of cross-immunity experiment*.—Although an infection with *P. inui* var. *cynomolgi* developed, no disturbance of health or extensive parasitic invasion was found. In evaluating this experiment, it must be remembered that

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\* All the other experiments summarized here were carried out with *S. rhesus*, a species of monkey which is apparently more susceptible than *S. sinicus* to the pathogenic effects of *P. knowlesi*.

*S. irus*, as obtainable in India, appears to have a considerable immunity or tolerance to the pathogenic action of both these species of *Plasmodium*. Whether this tolerance is due to a natural immunity in this species of monkey, or whether it is the result of a tolerance acquired on account of previous infections in the natural state, it is impossible to say.

(v) Monkey No. 80. (*S. rhesus*).

*History of primary infection.* (*P. knowlesi* 'K<sub>1</sub>' strain). Severe attack which recovered with treatment [vide experiment (a)].

*History of superinfection.* Superinfected on 126th day with *P. inui* var. *cynomolgi* ('Cyn,' strain); parasites detected on 131st day and increased rapidly to a heavy parasitic infection on 134th, 135th and 136th days; no severe constitutional symptoms observed; spontaneous recovery; subsequently a chronic low-grade infection persisted, showing both species of *Plasmodium*, up to 206th day.

*Result of cross-immunity experiment.*—A superinfection with *P. inui* var. *cynomolgi* caused a severe attack of malaria due to this parasite. In contradistinction to the previous experiment, *S. rhesus* appears to have no natural tolerance to either of the two parasites used.

**(2) *P. knowlesi* superinfected on *P. inui* var. *cynomolgi*.**

(w) Monkey No. 93 (*S. rhesus*).

*History of primary infection.* (*P. inui* var. *cynomolgi*, 'Cyn,' strain). Parasites detected on 5th day and increased gradually, causing acute parasitic infection (without serious constitutional disturbances) reaching maximum on 12th day; no treatment given; spontaneous recovery; parasites present daily up to 28th day.

*History of superinfection.* Superinfected on 29th day with *P. knowlesi* ('C' strain); parasites detected on 43rd day; acute attack developed and monkey died of acute pernicious attack on 46th day; no treatment given; at time of death parasites almost exclusively *P. knowlesi*.

*Result of cross-immunity experiment.*—An infection with *P. inui* var. *cynomolgi* produced no immunity to a superinfection with *P. knowlesi*, which resulted in death.

**Summary of experiments on cross-immunity between *P. knowlesi* and *P. inui* var. *cynomolgi*.**

No evidence of cross-immunity could be demonstrated between these two species, when a susceptible animal was infected. The superinfection of *S. irus* by *P. inui* var. *cynomolgi* occurred in the presence of a latent infection with *P. knowlesi*. The clinical effects of both the primary and the secondary infections were slight in this case.

The number of experiments recorded is small and it is, therefore, impossible to draw any definite conclusions. The results suggest that no cross-immunity is produced between these two species of monkey Plasmodia.



TABLE A.  
Summary of superinfection and cross-immunity experiments.

Primary strains.	SUPERINFECTING STRAINS.					
	'C' strain.	'K <sub>1</sub> ' strain.		'K <sub>2</sub> ' strain.	'K <sub>3</sub> ' strain.	'K <sub>4</sub> ' strain.
'C'	Exp. (a) —	Exp. (h)	++ (SR)	Exp. (l)	+++ (RT)	Exp. (u) +++ (D)
	Exp. (b) ±	Exp. (i)	+++ (D)	Exp. (m)	+++ (D)	
	Exp. (c) —	Exp. (j)	+++ (RT)			
		Exp. (k)	+++ (RT)			
'K <sub>1</sub> '	Exp. (o) + + + (D)	Exp. (d) —		....	....	....
'K <sub>2</sub> '	Exp. (p) + + + + (D)					
	Exp. (q) + + + + (D)	Exp. (r) + + (SR)		Exp. (e) ±	....	....
'K <sub>3</sub> '	....	Exp. (s) + + (RT)		....	....	Exp. (v) + + (SR)
'K <sub>4</sub> '	....	Exp. (t) + + + (D)		....	Exp. (f) —	....
'Cyn <sub>1</sub> '	Exp. (w) + + + + (D)	....		....	....	Exp. (g) ±

*Explanatory Note:*—Exp. (a) refers to experiment (a) in the protocols, and similarly other letters given in brackets denote the corresponding experiments.

— indicates no attack or appreciable increase in the number of parasites.

± indicates a slight transient increase in the number of parasites.

++ indicates a definite increase of parasites, but without the production of clinical symptoms

+++ indicates an attack of moderate severity.

SR indicates a very severe attack.

RT means spontaneous recovery.

D means recovery with treatment.

D means death.

### GENERAL DISCUSSION.

The results of each series of experiments have already been discussed in the summaries given previously. It is felt that the experimental work has not yet proceeded far enough to justify any more definite deductions being made at this stage, apart from those already given.

It is interesting, however, to compare some of the results obtained, with those reported by other workers on avian, human and simian malaria. As in the latter work on superinfection with different strains of *Plasmodia*, we have found that a chronic or latent infection produces an immunity or tolerance to superinfection with the same strain of parasite.

The work on avian malaria suggests that, with *P. relictum* at least, a heterologous tolerance is produced to different strains of the same species of parasite. On the other hand, in human malaria the evidence so far available suggests that no such heterologous immunity exists, at least with *P. vivax*. Immunity to the parasites of simian malaria appears to be very similar, in this respect, to that found in the case of the human plasmodial infections.\* These findings would appear to indicate that, at least in so far as superinfection with heterologous strains is concerned, simian malaria is more suitable for a comparison with human malaria than are avian infections.

As reported in both human and avian malaria, infections with one species of *Plasmodium* appear to confer no marked immunity to cross infection with another species of the same genus.

### SUMMARY.

The results obtained, under the conditions of our experiments, have been given in Table A. These may be summarized as follows:—

(1) Several strains of *P. knowlesi*, with different immunological characters, have been isolated from the Malayan monkey, *S. irus*.

(2) A chronic or latent infection with one strain of *P. knowlesi*, or with one strain of *P. inui* var. *cynomolgi*, appears to confer an effective immunity against the clinical effects of superinfection with the same strain of parasite.

(3) A chronic or latent infection with one strain of *P. knowlesi* does not confer any effective immunity against the occurrence of an acute attack of malarial fever, produced by a superinfection with a different strain of the same parasite.

(4) A chronic or latent infection with one strain of *P. knowlesi* appears to confer some tolerance to the clinical effects of superinfection with a different strain of the same parasite. This is indicated by an increased tendency for the initial acute attack of the superinfection to recover spontaneously, and by a diminished tendency for such infections to relapse at a later date.

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\* We have been unable to determine whether the immunity to superinfection with *P. brasilianum*, mentioned by Taliaferro (1932), refers only to homologous strains or not.

(5) The immunity produced by a given strain of parasites appears to be mainly specific for the same strain. There is, however, some evidence to suggest that a slight degree of common immunity exists between strains of the same species of parasite. This is possibly non-specific and due to a general stimulation of the reticulo-endothelial system by such malarial infections.

(6) The results of our experiments suggest that any effective immunity developed against one special strain of parasite, is not due merely to such a general stimulation of the reticulo-endothelial system, but that some undetermined specific factor plays a very important rôle in this connection.

(7) No cross-immunity has been demonstrated between infections due to *P. knowlesi* and those due to *P. inui* var. *cynomolgi*. The number of experiments is, however, too few upon which to draw any fixed conclusions.

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## ABSTRACTS.

As was pointed out by Sinton (1929), much valuable work in relation to malaria in India remains unheard of, except by a very limited number of people. The records of such work are, in many instances, either published locally or remain as manuscript reports buried in the files of local offices, and thus are not included in any bibliography of the subject. This state of affairs is due in most cases to financial stringency, which makes publication impossible, or to the fact that many of the reports are made up of data, which is mainly of local interest only. In the first number of the *Records of the Malaria Survey of India*, an attempt was made to compile a bibliography, which would include reference to many of the reports of this nature that had been compiled in the past, thus saving them from oblivion, and bring them to the notice of Indian workers.

It was hoped at one time that it would be possible to publish many of these reports in full in this journal, but this has been found financially impossible. It has, therefore, been decided to publish abstracts of such papers, giving prominence to the salient features and more important observations, especially those of more general interest, while copies of the originals are placed for reference in the Library of the Malaria Survey of India. In this way it is hoped that valuable data will be placed permanently on record, and that local workers will in future be able to obtain the loan of copies of the originals. This should preserve much information and many important records, which might be forgotten or lost in future years.

If Indian workers would send copies of papers of this nature to the Editor, an attempt will be made to publish abstracts of these in future numbers of the journal.

EDITOR.

## REFERENCE.

SINTON, J. A. (1929) .. .. *Rec. Mal. Surv. Ind.*, 1, 1, pp. 1-3.



REPORT ON A MALARIA SURVEY IN KALIMPONG AND SIKKIM.\*

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(MS. 29 typed pages and 2 Maps.)

[December 5, 1932.]

THIS report is based on investigations carried out in the Kalimpong Sub-division of the Darjeeling District, Bengal, and in Lower Sikkim, between 1st July and 8th August, 1932. Details are given regarding the topography, physiography, climate, population, vital statistics, and agricultural conditions in the areas surveyed, and also of the social conditions and habits of life of the inhabitants. The data collected to indicate the incidence of malaria and its mode of transmission, consist mainly of spleen and blood examinations among children from 2 to 10 years of age, and a study of the Anopheline fauna, their habits and breeding places. Recommendations are made for the amelioration of malaria in those parts where the disease is endemic, and for the prevention of its spread to healthy localities.

The report deals separately with two main areas, namely, (a) Kalimpong Subdivision, and (b) Sikkim.

\* The original report is available for consultation in the Office of the Director of Public Health, Bengal, and in that of the Director, Malaria Survey of India, Kasauli.



*(a) Kalimpong Subdivision.*

The area surveyed is situated among the foothills of the eastern Himalayas, and is almost entirely mountainous, ranging in altitude from about 600 to almost 5,000 feet above sea-level. The climatic conditions are therefore very divergent. In the low-lying areas in the vicinity of the Tista and Rilli rivers the climate at this season is hot and humid, while at higher elevations it is more temperate. Villages are scarce and the population, which is scanty, is scattered in small holdings over the hillsides, on which maize, millet and rice are grown on terraces. Irrigation is from 'jhoras' or springs, the water being conducted to the fields in contour channels, or in bamboo pipes. A large part of the country is untilled and is covered with jungle in which streams, springs and seepages are very numerous.

Besides the town of Kalimpong (4,000 to 4,500 feet), sixteen villages, varying in elevation from 625 to 4,700 feet, were surveyed. The spleen, parasite and mosquito findings have been summarized in Table I. The authors note that the incidence of malaria varies with the altitude, the spleen rates being highest at the lower elevations, and negligible above 4,000 feet. Between 2,000 and 3,500 feet the incidence of malaria is comparatively slight, but definite evidence of local transmission was obtained. In the authors' opinion, this is facilitated by the introduction of gametocyte carriers from the more malarious places at lower levels, *e.g.*, coolies returning from road and railway construction work.

Eight species of *Anopheles* were found either as adults or as larvæ. These species are enumerated, and the localities from which they were collected are indicated in Table I, which has been compiled from the authors' figures.

*(b) Sikkim.*

The part of Sikkim surveyed includes (1) Gangtok, the capital, and four neighbouring villages, and (2) thirteen villages in the vicinity of the Tista River. The nature of the country, the population and the agricultural conditions are similar to those in the Kalimpong area. Within recent years the population has increased considerably as the result of immigration of Nepalese and Tibetans who have settled as cultivators. The altitude of the places surveyed varied from 1,200 to 5,700 feet above sea-level, and, as in the case of the Kalimpong area, the incidence of malaria appeared to vary inversely with the altitude. Details of the spleen, parasite and mosquito findings are given, and these have been summarized in Table II.

The authors believe that *A. maculatus* is the vector mainly responsible for the transmission of malaria in both the Kalimpong and Sikkim areas. This belief is based chiefly on the prevalence of this species, and on its reputation as a carrier in Malaya. The principal breeding places of *A. maculatus* were found to be slow running streams and 'jhoras', seepages and terraced paddy fields. The apparent absence of adults of this species from human habitations,

TABLE I.  
Summary of spleen, parasite and anopheline findings in Kalimpong Subdivision.

Name of town or village.	Approximate height in feet above sea-level.	SPLEENS.				PARASITES.		ANOPHELES.					
		Number of children examined.	Number with enlarged spleens.	*Percentage spleens with enlarged spleens.	Number of children examined.	Number with malaria parasites in blood.		ADULTS.					
								<i>A. maculatus</i> .	<i>A. maculatus</i> var. <i>willmori</i> .	<i>A. subpictus</i> .	<i>A. vagus</i> .	<i>A. culicifacies</i> .	<i>A. maculipalpis</i> .
								<i>A. maculatus</i> var. <i>willmori</i> .	<i>A. subpictus</i> .	<i>A. vagus</i> .	<i>A. culicifacies</i> .	<i>A. maculipalpis</i> .	<i>A. lindesayi</i> .
								<i>A. maculatus</i> + <i>willmori</i> .	<i>A. subpictus</i> .	<i>A. vagus</i> .	<i>A. culicifacies</i> .	<i>A. maculipalpis</i> .	<i>A. lindesayi</i> .
Rivang ..	625	11	3	27.2	5	3		..	..	1	..	..	..
20th mile Basti from Siliguri.	675	10	1	10.0	2	1		..	..	1	..	..	..
Giellekhola ..	675	26	9	34.6	5	2		..	..	12	..	..	..
Tista Bazar ..	710	115	25	21.7	28	8		..	..	2	..	..	..
Melli ..	800	9	2	22.2	2	1		..	..	4	..	..	..
Tarkhola ..	1,000	22	17	77.7	17	4		..	..	..	..	..	..
Takling ..	1,510	4	1	33.3	4	1		..	..	..	..	..	..
Chibo ..	2,000	35	4	11.4	1	..		..	..	..	..	..	..
Sortang Forest Basti ..	2,000	16	1	6.2	6	..		..	..	..	..	..	..
Mangwa ..	2,500	12	2	20.0	2	..		..	..	..	..	..	..
Bong ..	2,500	29	2	6.8	7	1		..	..	10	..	..	..
Sindipung ..	2,500	41	5	12.1	5	5		..	..	3	..	..	..
Dungra ..	3,000	63	2	3.1	7	1		..	..	15	..	..	..
Sortang Basti ..	3,500	15	..	0.0	..	..		..	..	..	..	..	..
Simlay ..	3,000	25	2	8.0	3	1		..	..	..	..	..	..
Kalimpong ..	4,000	448	11	2.4	58	3		69	1	25	..	..	75
Mungpoo ..	4,500	..	..	..	..	..		..	..	..	..	..	..
	4,700	48	..	0.0	..	..		..	..	..	..	..	..

\* The numbers given in some cases are too small to give a reliable percentage.

† Details of the different types of infection are given in the original paper.

TABLE II.

Summary of spleen, parasite and anopheline findings in Sikkim.

Name of town or village.	Approximate height in feet above sea-level.	Spleens.				Parasites.		ADULTS.						LARVÆ.								
		Number of children examined.	Number with enlarged spleens.	Spleen rate.	Number of children examined.	Number with parasites in blood.	<i>A. maculatus</i> var. <i>willmori</i> .	<i>A. vagus</i> .	<i>A. hyrcanus</i> var. <i>nigerrimus</i> .	<i>A. fuliginosus</i> .	<i>A. culicifacies</i> .	<i>A. aikenii</i> var. <i>bengalensis</i> .	<i>A. maculatus</i> + var. <i>willmori</i> .	<i>A. vagus</i> .	<i>A. hyrcanus</i> var. <i>nigerrimus</i> .	<i>A. fuliginosus</i> .	<i>A. maculipalpis</i> .	<i>A. lindesayi</i> .	<i>A. aikenii</i> var. <i>bengalensis</i> .	<i>A. gigas</i> .		
Rungpo	1,200	24	13	54.1	8	6	5	1	40	1	..	4	..	31	21	11	7	..	..	..	..	..
Kopchu	1,200	4	3	75.0	3	3	..	7	..	..	..	..	..	49	2	..	..	..	..	..	..	..
Majhar	1,200	47	38	80.8	20	17	52	..	7	..	..	..	..	..	..	..	14	..	..	..	..	..
Khangson	1,300	13	12	82.3	12	6	..	..	..	..	..	..	..	29	3	..	..	..	..	..	..	..
Sankathola	1,400	22	17	77.1	15	11	10	..	60	..	..	..	..	150	25	1	..	..	..	..	..	..
Singtam	1,400	49	42	85.0	31	21	1	..	11	..	..	..	..	41	..	..	..	10	..	..	..	..
Monring	1,500	8	7	87.5	5	1	..	..	..	..	..	..	..	7	..	..	..	..	..	..	..	..
Sirhamy-basar	1,600	8	8	100.0	6	3	5	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Reshob	2,000	6	6	100.0	6	5	5	..	..	..	..	..	..	10	1	..	..	..	..	..	..	..
Duga	2,500	27	7	25.9	7	4	1	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Tokal	3,000	34	25	73.5	14	10	..	..	..	..	..	..	..	14	1	..	..	..	..	..	..	..
Temi	3,000	12	6	75.0	11	5	..	..	..	..	..	..	..	16	..	..	..	..	..	..	..	..
9th mile from Gangtok.	3,000	4	3	75.0	4	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
8th mile from Gangtok.	3,000	11	6	54.5	7	6	5	..	1	..	..	..	..	1	..	..	..	..	..	..	..	..
Taktu	3,300	15	11	73.3	12	2	..	..	..	..	..	..	..	16	5	..	..	..	..	..	..	..
Tedong	3,700	16	5	31.2	5	3	5	..	4	..	..	..	..	..	..	..	..	..	..	..	..	..
Shayyong	3,700	21	3	14.2	3	2	14	1	7	..	5	..	..	..	..	..	..	..	..	..	..	..
Gangtok	5,700	63	0	0.0	16	0	27	1	29	..	..	..	..	38	..	..	3	..	..	..	..	3

cowsheds, etc., was at first puzzling and against the theory that it might be the principal vector. Experiments, however, showed that *A. maculatus* enters houses after sundown and feeds freely on both man and domestic animals, but leaves again before day-break. It was found to shelter during the day in the shade of hillside vegetation, in caves, under bridges, etc.

The following numbers and species of *Anopheles* were dissected in the localities indicated, but none was found infected :—

Kalimpong area.		Sikkim area.	
<i>A. maculatus</i>	.. 61	<i>A. maculatus</i>	.. 83
<i>A. maculatus</i> var.		<i>A. maculatus</i> var.	
<i>willmori</i>	.. 28	<i>willmori</i>	.. 28
<i>A. maculipalpis</i>	.. 1	<i>A. vagus</i>	.. 19
<i>A. lindesaii</i>	.. 1	<i>A. culicifacies</i>	.. 2
<i>A. culicifacies</i>	.. 1		

In the opinion of the authors any measures directed towards the eradication or control of breeding places in the areas surveyed would be impracticable for financial reasons. The adoption of biological measures did not appear to them to afford a solution of the problem. They therefore recommend quininization of the population in the malarious tracts. Quinine should be combined with plasmoquine to ensure the destruction of gametocytes.

The town of Gangtok in Sikkim is considered to be free from malaria, and likely to remain so. Kalimpong town, on the other hand, is regarded as a potentially malarious locality. Although at present there is no evidence of endemic malaria in this town, the climatic conditions appear to be favourable for transmission, and *A. maculatus* is prevalent. It is thought that with the advent of motor transport and the consequent introduction of gametocyte carriers from less healthy areas, all the necessary factors for the spread of malaria will be supplied. The authors therefore recommend that wet cultivation should be prohibited within a radius of  $\frac{1}{2}$  mile of the town, or, failing this, that irrigation should be regulated so that water will not stand in the fields for periods exceeding one week.

H. W. M.



**REPORT ON A BRIEF SURVEY OF MALARIA AND ANOPHELINES IN  
PATNA.\***

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(MS. 7 pp., two charts, two photographs and 1 Map.)

[January 23, 1933 ]

A BRIEF survey of the Anopheline fauna and the prevalence of malaria in Patna, the capital of Bihar and Orissa, was made during a period of 8 days in October 1930. The author's personal observations were, therefore, very limited, but are nevertheless of interest in view of the paucity of such data from this area.

Information is given with regard to the topography and climatic conditions of Patna. The town itself, including the city and the new capital, is situated on relatively high ground along the south bank of the Ganges River, and occupies an area of about 15 miles long and from  $\frac{1}{2}$  to  $1\frac{1}{2}$  miles broad. Owing to the natural slope of the town towards the south, drainage of storm and surface water is away from and not towards the River, resulting in the formation of swamps and marshes to the south. One of these swamps extends for a distance of eight miles and formerly communicated with the river but is now cut off by the deposition of silt. These swamps and marshes form ideal breeding places for Anopheline mosquitoes as do also the drains and borrow-pits associated with the railway and brickfields, which are also situated to the south of Patna. The water supply is partly from wells and partly piped. In the latter case the water is raised from tube wells to overhead reservoirs, and

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\* The original report is available for consultation in the Office of the Director, Malaria Survey of India, Kasauli.

is distributed by gravity. As the piped supply is intermittent, concrete 'chaubachas' for storage are common.

#### *Anopheline fauna.*

The swamps and marshes to the south form ideal breeding places for Anopheline mosquitoes. The following numbers and species of adult Anophelines were identified :—

<i>A. barbirostris</i>	..	..	..	4
<i>A. culicifacies</i>	..	..	..	14
<i>A. fuliginosus</i>	..	..	..	14
<i>A. hyrcanus</i>	..	..	..	12
<i>A. subpictus</i>	..	..	..	254
<i>A. vagus</i>	..	..	..	44

The author emphasizes the absence of *A. stephensi* from the town in spite of the presence of apparently suitable breeding places, such as wells and 'chaubachas'. This species was specially looked for on account of its prevalence in some other large towns in India, and its notoriety as an urban malaria carrier.

#### *The prevalence of malaria in Patna.*

Owing to the brevity of the survey few original data were collected. Routine blood examinations of local children were not attempted, but the spleen census was taken in two areas (Kadam Khau and Patna City). Eighty children were examined in the former area and thirty-seven in the latter, the spleen rates being 6 and 10 per cent respectively. The author points out that the spleen rate is not an absolutely true index of malaria in this area owing to the presence of kala-azar.

Statistics of malaria cases in local hospitals and dispensaries for the year 1929 are given. The bloods of 26 patients, diagnosed clinically as malaria, were examined and of these 15 showed parasites (*P. vivax* and *P. falciparum*) in the blood, *P. falciparum* being the predominant species.

#### *Transmission of malaria in Patna.*

The author believes that *A. culicifacies* and *A. fuliginosus* are the species responsible for local malaria transmission. This opinion is based on the reputation of these two species as malaria carriers in other parts of India.

The opinion is expressed that there is danger of the appearance of malaria in Patna in epidemic form, and that a more detailed investigation is necessary.

H. W. M.

## SOME OBSERVATIONS ON THE MOSQUITOES AND SANDFLIES OF RAJPUTANA.\*

BY

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(MS. 13 pp.)

[April 22, 1933.]

THE paucity of records relating to the Anopheline fauna in Rajputana led to the author undertaking two short tours in this area, with the object of obtaining more information on this subject. At the same time the opportunity was taken to collect Culicine mosquitoes and sandflies.

The report gives much information with regard to the population, the living conditions and occupations of the inhabitants, the nature of the country, and other points of local importance. The knowledge gained regarding the mosquito and sandfly fauna in this region is of great general interest and importance, as little was previously known on this subject.

A brief summary of the conditions prevailing in the various places visited, and a list of the mosquitoes and sandflies† collected are given below :—

### (a) *Sambhar town and Salt Lake.*

This area was visited between 26th May and 3rd June, 1932, i.e., during the hot dry season of the year. Sambhar town (1,200 feet above sea-level) lies in a flat arid zone of sandy, unfertile country on which little or no jungle grows. The main industry is the manufacture of salt from the Salt Lake which covers an area of 90 square miles, and the water of which has a high saline content during the hot dry months.

No mosquito-breeding was found to occur in the Salt Lake itself, but larvæ were recovered from a number of other places (waste water pools, unused wells, garden tanks, etc.).

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\* The original MS. of this report has been filed in the Library of the Malaria Survey of India, Kasauli, and is available for reference by anyone who wishes to obtain more detail of local interest.

† The Culicine mosquitoes collected were identified by Captain P. J. Barraud, F.E.S., F.E.S., F.L.S., Entomologist, Malaria Survey of India, Kasauli. The sandflies collected were identified by Lieutenant-Colonel J. A. Sinton, V.C., M.D., D.Sc., I.M.S., Director, Malaria Survey of India, Kasauli.



The following species of Anopheline mosquitoes, caught as adults or bred from larvæ, were recorded :—

<i>A. stephensi</i> .	<i>A. annularis</i> ( <i>A. fuliginosus</i> ).
<i>A. subpictus</i> .	<i>A. culicifacies</i> .

An interesting observation was the finding of the larvæ of *A. stephensi* in unused wells containing brackish water, and in a 'Bauri' containing clear, brackish water.

*Culex fatigans* and *Culex vishnui* were also collected in this area.

The following species of *Phlebotomus* were recovered :—

*P. argentipes*, *P. colabaensis*, *P. sergenti*, *P. babu* and *P. baghdadis*.

#### (b) Mount Abu.

Two short visits were made to this area, the first from 11th to 20th June, 1932 (dry season), and the second from 5th to 26th October, 1932 (after the monsoon). Mount Abu is an isolated peak of the Aravalli range. It is the only mountain in Rajputana rising to a height of over 4,000 feet above sea-level. The hill-sides are rich in vegetation and are covered with areas of fairly dense forest, interspersed with stretches of bamboo jungle. The climate is dry for the greater part of the year, but very wet during the monsoon. The rainfall varies from 56 to 100 inches per annum. The flat summit of the hill is the site of the town of Abu, a hill station, composed of a cantonment, palaces of the Ruling Chiefs of the Rajputana States, official residences and a bazaar.

Mosquito breeding places include hill streams, seepages, tanks, pools in nullahs, borrow-pits, holes in rocks, etc.

The following species of Anopheline mosquitoes, caught as adults or bred from larvæ, were identified :—

<i>A. annularis</i> ( <i>A. fuliginosus</i> ) (June: October).
<i>A. culicifacies</i> (June: October).
<i>A. fluviatilis</i> ( <i>A. listoni</i> ) (June: October).
<i>A. moghulensis</i> (October).
<i>A. subpictus</i> (June: October).
<i>A. splendidus</i> ( <i>A. maculipalpis</i> var. <i>indiensis</i> ) (October).
<i>A. jamesii</i> (October).
<i>A. stephensi</i> (October).
<i>A. theobaldi</i> (June: October).

The Culicine mosquitoes collected were identified as :—

<i>C. fatigans</i> .	<i>Aedes</i> ( <i>F.</i> ) <i>pseudotaeniatus</i> .
<i>C. vishnui</i> .	<i>Aedes</i> ( <i>S.</i> ) <i>albopictus</i> .
<i>C. barraudi</i> .	<i>Aedes</i> ( <i>S.</i> ) <i>w-albus</i> .
<i>C. bitaeniorhyncus</i> .	<i>Aedes</i> ( <i>S.</i> ) <i>unilineatus</i> .
<i>C. (Lutzia) fuscans</i> .	<i>Aedes</i> ( <i>Diceromyia</i> ) <i>iyengari</i> .
<i>C. (Lutzia) raptor</i> .	<i>Aedes</i> ( <i>C.</i> ) <i>thomsoni</i> .
<i>C. fuscocephalus</i> .	<i>Heizmannia chandi</i> .

The only *Phlebotomus* found was *P. sergenti*.

(c) *Ajmer city.*

Ajmer, a large and important city in Rajputana, was visited between 3rd and 11th June, 1932 (the hot dry season of the year). The city is situated on a flat, arid plain about 1,500 feet above sea-level. This plain is surrounded by the low hills of the Aravalli range. The soil of the plain is shallow and mostly sandy. Beneath this is a rocky stratum which rises close to the surface, or protrudes above it as bare rock. With the exception of the limited areas under cultivation, there is little or no vegetation in the vicinity of the city. Rainfall is scanty and uncertain averaging 21·2 inches per annum. Irrigation is from wells.

Mosquito breeding was found to occur in large tanks, beds of 'kutchas' drainage nullahs, collections of water into which these nullahs discharge, leakages from water hydrants, etc.

In spite of the unfavourable season of the year, the following species of Anopheline mosquitoes were found, either as adults or bred from larvæ :—

*A. subpictus.*

*A. annularis* (*A. fuliginosus*).

*A. stephensi.*

*A. pulcherrimus.*

*A. culicifacies.*

The Culicine mosquitoes collected were :—

*Aedes aegypti* (*Stegomyia fasciata*).

*Culex fatigans.*

The following species of *Phlebotomus* were caught :—

*P. sergenti.*

*P. baghdadis.*

*P. papatasii.*

*P. minutus* var. *antennatus.*

*P. colabaensis.*

*P. bailyi* var. *campester*

*P. clydei.*

*P. argentipes*

*P. babu.*

H. W. M.



## MALARIA AND WATER-LOGGING.

BEING

### A REPORT ON A STUDY OF THE HEALTH CONDITIONS PREVAILING AT CHAKANWALI, GUJRANWALA DISTRICT, PUNJAB, FROM NOVEMBER 1927 TO JANUARY 1931, WITH SPECIAL REFERENCE TO MALARIA IN A WATER-LOGGED TRACT.

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[April 15, 1933.]

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## I. INTRODUCTORY.

THE Punjab, which constitutes the north-west portion of the Great Indo-Gangetic Plain, is traversed from north-east to south-west by five great rivers from which it derives its name of 'the Land of Five Waters'. It stretches from the foot of the Himalayas in the north to the desert of Rajputana in the south. Between the rivers are strips of country known as the '*doabas*', which are at a slightly higher level than the riverains. The world-famous irrigation system of the Punjab was conceived with the idea of irrigating the *doabas*. The tract of country, with which this report is concerned, is situated in the *Rechna Doaba* which is the name given to the strip of country lying between the Ravi and the Chenab rivers.

Before the advent of canal irrigation, the *Rechna Doaba* presented considerable variations in regard to the nature of the soil, the level of subsoil water and density of population as one travelled from north-east to south-west. Thus the well-settled, fully-developed north-western tract (locally known as the '*charkhari*') with a rich soil, a relatively high subsoil water level (20—30 feet) and extensive well irrigation gradually gave place further south to a less productive tract ('*bangar*'). Here the soil was lighter, the subsoil water level lower (25—45 feet) and the population less dense. Irrigation was carried out from wells. This method was not profitable, unless supplemented by rain. The *bangar*, in its turn, merged towards the south into a waterless almost rainless and unculturable tract known as the '*bar*'. The subsoil water level in this

area was extremely low (40—75 feet) and the cost of sinking and working wells was well-nigh prohibitive. The crops were solely dependent upon the rainfall, and the population was sparse and unsettled.

The Lower Chenab Canal was constructed in 1894 to irrigate the *Rechna Doaba* and more especially its *bar* and *bangar* tracts. It takes off from the Chenab river at Khanki (*vide* Map I), has a capacity of 11,000 cusecs and commands about two million acres of land. It runs in a deep cutting for the first 12 miles but gradually approaches the surface so that at Fatehpur, which is 15 miles below the headworks, it flows partly between embankments. As the canal passes along the Chakanwali Reclamation Farm (21 miles below Khanki) the head of water is 3 to 4 feet above the ground level when the canal is full. The main canal runs in the south-westerly direction for a distance of 28 miles until it reaches the uplands, or the watershed between the Ravi and the Chenab rivers, where it divides at Sagar into two large branches which feed the *bar* area. Since the opening of the canal the aspect of the country has entirely changed. The vast barren tracts of the *bar* which were formerly shrub lands, have given place to miles of smiling fields which yield bumper crops and support a prosperous and growing population. The canal, however, has not proved an unmixed blessing. Soon after it became a permanent waterway, the subsoil water level in its immediate vicinity began to rise in parts of the *bangar* tract. At first it proved beneficial to the cultivators who were able to work their wells with less labour. Later, about 1904, the evil effects of water-logging began to be noticed and in certain parts field after field went out of cultivation. In 1927, 43,746 acres of land were estimated to have suffered on account of seepage from the canal and its branches. Here no crop, except rice, could be raised and that too only in restricted areas. The soil in many parts of these localities was salt-bearing, *i.e.* 'kalraṭi', and, with the rise in the level of the subsoil water, saltpetre efflorescence appeared on the surface, thus rendering cultivation still more difficult. At the same time along the canal, seepage water appeared on the surface in many places and extensive swamps formed in low-lying areas.

Remedial measures, such as the provision of a waterproof lining of the channel, the digging of drains parallel to the canal and the opening up of the natural drainage were carried out. To a certain extent they reduced the amount of surface water, but they failed to lower the level of the subsoil water. The Chakanwali Reclamation Farm is situated in one of these unfortunate tracts where, on account of water-logging, misery and desolation have followed in the wake of the prosperity produced by canal irrigation. In the year 1922 the landholders of Chakanwali village represented to the Director of Public Health the pitiable condition to which they were reduced, and Lieut.-Col. C. A. Gill, I.M.S., then Assistant Director of Public Health, Punjab, was detailed to investigate the complaint. He visited Chakanwali and neighbouring villages in August 1922, and carried out a malaria survey. In his report (Gill, 1922), he states that Chakanwali was completely surrounded by water and could only be

approached with difficulty, in spite of the fact that there had been no heavy rains for some weeks. In the outskirts of the village the water in many of the wells was flush with the surface. The parasite and the spleen rates were extremely high. The people complained of the scarcity of both food and fodder. Colonel Gill came to the conclusion that the disease responsible for the insalubrity of the tract was undoubtedly malaria of a chronic cachectic type. This he attributed to the fact that, under the extremely adverse economic conditions, the recovery of infected individuals was retarded and many relapses occurred. He emphasized that the situation was serious and required urgent action on the part of the Irrigation Department.

The report of Colonel Gill was considered by the Drainage Board on the 6th December, 1922, and it was then that Chakanwali first came into prominence. At this meeting there were differences of opinion in regard to the claims of the villagers to compensation. It was eventually decided to watch for a period of two years the effect of the drains recently dug, and in the meantime to analyse the vital and agricultural statistics of the tract. This system of drains consisted of two open channels about 3 feet wide and 3 feet deep running parallel to the right bank of the canal and at distances of 400 feet and 800 feet respectively from it. It was later found that whilst they were partially effective in carrying off surface water, they made little impression on the saturation of the soil at short distances from them.

From this time onwards the evils of water-logging, due to canal irrigation, in this and other tracts presented serious problems for consideration by the Government. It became evident that a thorough scientific study of the problem of water-logging and of the measures necessary to prevent its occurrence and to reclaim water-logged land would have to be undertaken. The Chakanwali area was selected as a suitable site for an intensive study of this nature. The Government ultimately acquired by exchange 3,645 acres of land belonging to the villages of Chakanwali, Bhangwan, Kot Jan Bakhsh, Paleh and Jhattanwali and constituted the area into a Government Farm henceforth known as the Chakanwali Reclamation Farm. It was placed under the charge of the Scientific Research Officer of the Irrigation Branch of the Public Works Department. The actual reclamation operations commenced in November 1926. These consisted of agricultural adjustment (*i.e.*, growing of particular crops) and of a system of open field drains or subsoil drains running at *right angles* to the canal. They were linked to the main drain either directly or by a system of minors. For subsoil drainage the method used primarily was 'mole drainage', which consisted of forcing a torpedo-shaped steel tool of about 4 inches diameter through the soil at a depth of about 3 feet. The long hole or 'mole' thus produced by the compression of the soil was expected to remain effective for a long period. The mole system, however, did not prove successful in this area owing to the nature of the soil. It was, therefore, replaced by tile drainage which being expensive could not be extensively used and was replaced eventually by a system of open field drains. Attempts to reclaim salt-bearing (Thur)

areas were made by treatment of the soil with finely-powdered gypsum and also by green manuring.

The Public Health Department was not informed of the inception of this scheme and unfortunately no records of the health conditions prevailing during the first year of the reclamation work, which would have been valuable for purposes of comparison, were obtained.

On the 17th October, 1927, Colonel Gill, in company with Mr. King, Financial Commissioner, and Mr. Wilsdon, Scientific Research Officer, visited the farm and observed the remarkable change in the conditions in the reclaimed area. To quote Mr. King, 'The area which a year ago was swamp is now quite dry and over a great part of land which formerly produced no crops there has been grown a most flourishing crop of sugar-cane. Tenants are eagerly coming forward to take up land at ordinary '*batai* rate'.'

Immediately after his visit, Colonel Gill decided that the opportunity afforded by this experiment should be taken advantage of and an intensive study of the health conditions made, in conjunction with the parallel investigations of the Irrigation and Agricultural Departments.

The study was entrusted to the writers who paid their first visit to Chakanwali on the 12th November, 1927. Since then 34 visits have been made at more or less regular monthly intervals. The investigation, which was completed in January 1931, embraced the villages of Kot Jan Bakhsh and Chakanwali, whilst Kalerwala, a village situated outside the area under reclamation, was selected as a 'control'. The main lines on which the investigation was conducted were laid down by Colonel Gill. A census of the three villages was first taken, and arrangements were made to study, at monthly intervals, the spleen and parasite rates of children and adults, and to maintain accurate records of vital statistics and changes of population amongst the tenants and the employees. Monthly observations on the species of anopheline mosquitoes and their prevalence were made, and the level of the subsoil water in selected spots was recorded. One month after the commencement of the investigation, at the instance of Colonel Gill, a dispensary was opened at Kot Jan Bakhsh under a medical officer who was responsible for collecting morbidity statistics of the selected villages. It is proposed in this report to record the results of these observations and to discuss in the light of the data, thus collected, the natural history of malaria in the tract, which it is believed may be regarded as representative of malaria of other water-logged areas in the Punjab associated with canal irrigation.

## II. PHYSICAL FEATURES.

### (1) GEOGRAPHY.

Chakanwali Reclamation Farm is a roughly quadrilateral area of 3,645 acres with three villages on the site, *viz.*, Chakanwali, Kot Jan Bakhsh and

---

\* *Batai* rate. The land is leased out to tenants, and the produce is equally divided between them and the owner.



**Paleh.** The last-named village had been entirely abandoned but was recently re-occupied by the labourers employed at the farm, which is situated along the right bank of the Lower Chenab Canal in the Akalgarh police circle of Wazirabad Tahsil, District Gujranwala. Chakanwali lies about six furlongs to its north-east, whilst Kalerwala, the control village, is situated outside the farm area at a distance of about 2 miles to the south-west of Kot Jan Bakhsh.

## (2) PHYSIOGRAPHY.

**(a) Topography.**—The farm is an alluvial plain about 700 feet above sea level with a gradual slope towards the west and south-west. It is almost devoid of large trees except for a belt of acacia trees along the canal banks. It is cultivated only in the part that has been reclaimed, the rest of the area is still barren due either to saline efflorescence (*kallar*) or water-logging.

The main crops are sugar-cane, maize, rice and wheat. Wet cultivation (rice) is carried out during the months of July, August and September.

The land under cultivation is intermittently irrigated by gravity from the canal and to a certain extent by lift from the open drains. Drinking water is derived from wells, of which there are two at Kot Jan Bakhsh, three at Kalerwala, and two at Chakanwali.

It is a quiet countryside, there being no industrial towns or places of pilgrimage in the vicinity and, except for the labour now employed at the farm, there is nothing to attract people from outside.

**(b) Watercourses.**—As has already been stated the Lower Chenab Canal runs in a south-westerly direction and forms the eastern and south-eastern boundary of the farm. The Gajargola distributary takes off from the main canal a few furlongs above the farm and, running in a south-westerly direction, separates the north-western quarter of the farm from the rest.

An elaborate drainage system is now maintained in the reclaimed parts of the farm (*vide* Map II). There are two fair-sized open drains running parallel to, and at a short distance from, the right bank of the Lower Chenab Canal. These are known as the Jhattanwali drains Nos. 1 and 2. They were, as already mentioned, originally dug by the Irrigation Department to intercept seepage water from the canal, but being overgrown with weeds, which retard the flow of water, they do not function satisfactorily.

The Kalerwala drain is an important drain. It is kept in good condition by the farm management. It originates in the heart of the farm from a tank or sump which is fed by a number of minor channels which carry the subsoil water from the north-western plots. It takes at first a westerly, and later a south-westerly, course. It receives the Chakanwali drain which drains the subsoil water from Chakanwali and the fields and ponds around the village, and the Kot Jan Bakhsh drains which drain the area north of Kot Jan Bakhsh and also village ponds. It discharges into *Wagh Nullah* about 5 miles from the farm. The portion of the farm west of Kot Jan Bakhsh is drained by two

drains on either side of the Gajargola Railway Station road. These discharge partly into the Kot Jan Bakhsh drains and partly into the Gillanwali drain. The latter starts near the railway line and, running along the south-western boundary of the farm, discharges into Kalerwala drain. The Wazirabad-Lyallpur branch of railway line cuts straight through the farm at its western boundary. It runs on an embankment pierced by culverts which, being small, interfere with the flow of surface water from the area lying towards the north-west.

**(c) Ponds and depressions in the immediate vicinity of the villages.—**

The large ponds and depressions holding water, which surrounded these villages on all sides, have gradually disappeared as a result of systematic drainage. Now there are no depressions near the test villages which should provide breeding ground for the mosquitoes. Near Kalerwala, the control village, however, there are two big ponds. One of these does not harbour mosquito larvæ, possibly on account of the abundance of natural enemies. A number of small depressions on the southern side are the main breeding places in the vicinity of the village.

**(d) The soil.**—The geological formation of the soil as determined by a 74 feet boring on a sandy *tibba* (raised ground) near village Paleh is as follows :—

First there is a 9 feet layer of clay and sand, then a 4 feet layer of *kankar* (concretions of calcium carbonate), then 21 feet of pure sand and lastly 29 feet of clay, etc. There is a good deal of salt, which consists of chlorides, carbonates, bicarbonates and sulphates of calcium, sodium and magnesium in varying proportions, in different places in the soil. With the rise of the water-table this has been brought to the surface and concentrated in the upper layers of the surface soil on which it forms the saline efflorescence known as *kallar*.

When the farm was taken over by Government, out of a total area of 3,645 acres, 2,260 acres were affected by *kallar*. The reaction of the soil in *kallar* areas is extremely alkaline, the pH in parts being 9.6 to 10.0, while in grassy areas and in cultivated areas it ranges from pH 7.6 upwards. In some parts the soil, being highly alkaline, is impermeable and consequently rain water stands on the surface for a considerable period. The occurrence of *kallar* in association with water-logging, constitutes a grave combination and the main object of the reclamation work at Chakanwali has been the discovery of a solution of this difficult problem.

**(3) CLIMATE.**

In the district of Gujranwala, as in most districts in the Central Punjab, there are four well-marked seasons, *viz.*, spring which lasts from March to May, summer from June to August, autumn from September to November, and winter from December to February. The average annual rainfall (1866-67 to 1893-94), as recorded at Hafizabad rain-gauge station, is 23.3 inches. The monsoon rains and the winter rains are both subject to considerable variation, the monsoon

being decidedly poor once in three years and the winter rains twice in every three years.

The Scientific Research Officer keeps a continuous record of the temperature and humidity at the farm, by means of an automatic thermograph and a hair hygrometer installed in Stevenson's screens in the open ground opposite the Farm Manager's office at Kot Jan Bakhsh. Daily maximum and minimum temperature and humidity figures for the period between December 1927 and December 1930 (excepting for a few months for which the records were missing) were abstracted from these records and charted.\*

A perusal of this chart brings out the following points :—

1. The temperature shows a wide range of fluctuation from below freezing point in the winter to above 120°F. in the summer.
2. The diurnal changes in the temperature are smallest during the winter, and to a certain extent during the rainy season. They are greatest during the spring and the autumn.
3. Sudden changes of temperature from day to day are very marked during the spring and the rainy season.
4. The seasonal fluctuations are comparatively less marked in the case of relative humidity and it is especially so with the maximum humidity which maintains a high value, viz., 70 per cent to 90 per cent throughout the year. The values for 1929 (March to May) are, however, distinctly lower than the corresponding months of 1928 or 1930.
5. The diurnal changes as well as sudden changes from day to day are, however, very well marked especially in the case of minimum humidity.
6. Rains considerably affect the minimum humidity but not the maximum humidity.
7. Monsoon rainfall in inches for the period under observation was as follows :—

Year.	July.	August.	Combined July and August.
1928	.. 1.88	8.02†	9.90
1929	.. 10.46	7.49	17.95
1930	.. 11.20	2.67	13.87

8. Total precipitation for the last 6 months of 1927 was 8.09 inches; for 1928, 12.16 inches; for 1929, 19.49 inches; and for 1930, 14.11 inches.

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\* The Editor regrets that it has not been possible to reproduce this very large chart. Copies are, however, available in the office of the Epidemiological Bureau, Punjab, Lahore, and the library of the Malaria Survey of India, Kasauli, for consultation by those interested.

† Of these 8.02 inches rainfall, 6.50 was recorded on 31st August and 1st September.

9. We are unable to explain why humidity in 1929 was so low, although rainfall was greater than during 1928 and 1930. The Scientific Research Officer doubts the reliability of the automatic hair hygrometer and probably instrumental defect is responsible for the discrepancy.

### III. RISE IN WATER-TABLE AND ITS EFFECTS.

As has been mentioned before, the *Rechna Doaba*, which is about 10,000 square miles in extent being roughly 160 miles long and 64 miles wide, is enclosed between the Ravi and the Chenab rivers. The subsoil water in this area was originally derived mostly from these rivers, and only a small part came directly from the Himalayas. The water-table, therefore, sloped towards the centre from either river and met somewhere in the middle of the *doaba*. When, however, the canals were dug, due to the head of water and capillary action, ridges of water-table formed, sloping from the canals towards the rivers. Ultimately the direction of the flow of subsoil water was reversed being now from the centre of the *doaba* towards the rivers. The extent to which the spring level was raised in different localities depended upon a number of factors, *e.g.*, the amount of seepage from the canals, obstruction to the natural drainage of the area, nature of the soil, distance from the river, etc.

It has been estimated that about 40 to 45 per cent of canal water goes to increase the subsoil water. The extent of seepage according to one irrigation expert depends upon three main factors, *viz.*:

- (1) concentration of large volumes of flow,
- (2) high elevation of flow, and
- (3) continuity of flow.

It has thus been found that the main Lower Chenab Canal causes a very high ridge in spring level compared to its distributaries.

Chakanwali Farm is particularly badly situated. It is very close to the main canal in which huge volumes of water continually run between raised embankments and from which seepage takes place at a high rate. The Gajargola distributary causes another ridge of the water-table and obstructs the natural flow of the subsoil stream towards the river. Surface water is held up between the two canals and the railroad embankment. The Jhattanwali drains help to carry away some of the surface water, but they are inadequate. We are, therefore, faced with a condition of saturation of the soil with more or less stagnant water. Chart II shows how the water-table has steadily risen in this area since the canal became a permanent water-way.

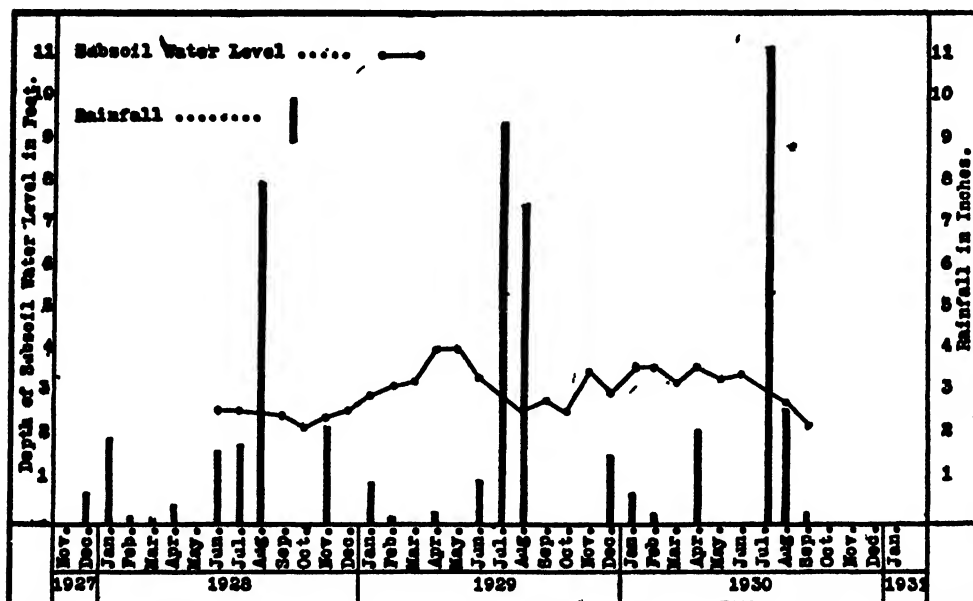
#### (1) THE WATER-TABLE.

The farm, which is situated in the worst part of what is known as the Hafizabad water-logged tract, has been water-logged at least since the year 1911.

During the period of investigation, observations on the fluctuation in the level of the subsoil water were taken systematically near the villages of Chakanwali and Kot Jan Bakhsh, by means of vertical pipes sunk for the purpose. The position of these pipes with respect to the villages is shown in Map II. Chart I shows the average depth of the subsoil water below the surface in pipe No. 5 of lines Nos. I, II, III and IV at the middle of each month.

CHART I.

Average depth of subsoil water below natural ground level taken in pipe No. 5 of lines I, II, III and IV in the middle of each month, also total rainfall during each month.



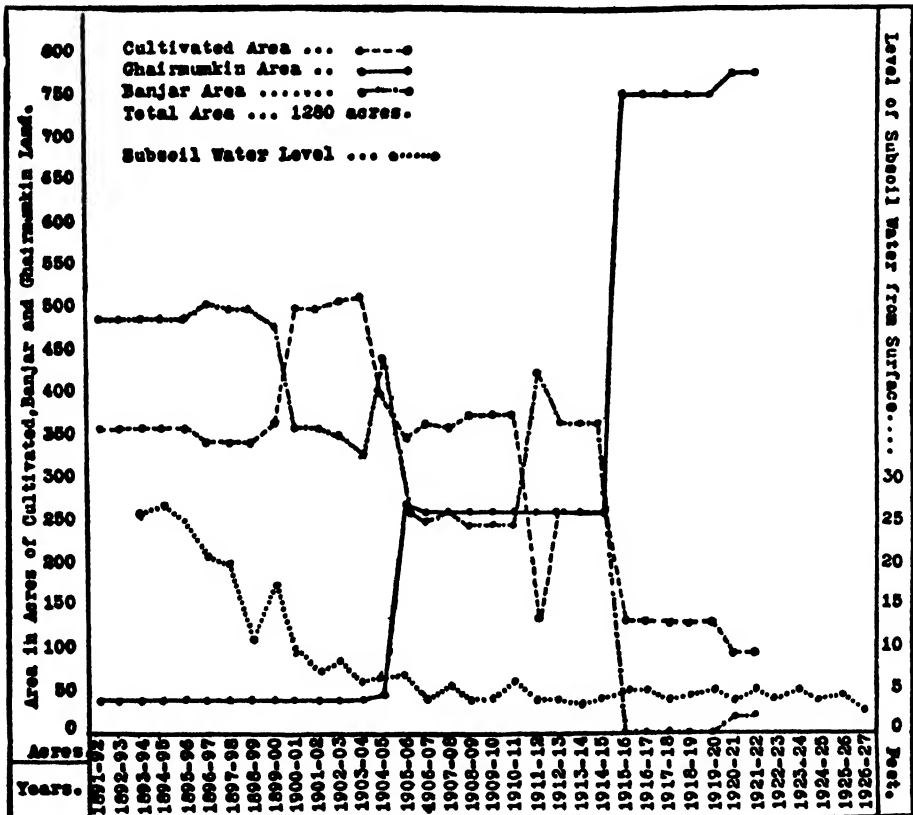
## (2) WATER-LOGGING AND AGRICULTURE.

To what extent the water-table should rise before the land could be considered as 'water-logged' from the agricultural point of view seems difficult to fix definitely. The definition, adopted by the Punjab Water-logging Conference of 1917 that 'It was a condition of the subsoil water-table which was injurious to agriculture and more or less deleteriously affected the public health', is nothing but a truism. Mr. C. H. Clayton, Chief Engineer of the Board of Agriculture and Factories, England, defined water-logging as 'any land, light or heavy, in which the saturation level or "Soc" is within 18 inches of the surface'. Others consider a rise in spring level within 5 feet of the surface as danger point. In March 1925 the Punjab Drainage Board laid down that land should be considered to be water-logged which, having been thrown permanently out of cultivation owing to rise in subsoil water, has been

entered as *banjar* in the revenue records, and beneath which the subsoil water is at or within 4 feet of the surface in the month of June. In practice this definition was not found to be satisfactory, as it failed to take into account the salinity of the soil which materially contributed to the damage caused by rise in subsoil water level. Another factor of importance which is being carefully examined by the Scientific Research Officer is in regard to the movement of subsoil water, for it has been shown that so long as the subsoil stream maintains a good flow a high water-table even up to two feet from the surface does not prevent the growth of good crops.

## CHART II.

Area in acres of cultivated, *banjar* and *ghairmumkin* land in village Chakanwali (1891-92 till 1921-22) and level of subsoil water in month of June (1891-92 till 1926-27).



As early as 1894, Mr. Michael O'Dwyer (now Sir Michael O'Dwyer) noticed a high saline content of the soil in this locality. He expressed his fear that with the rise of subsoil water level the salts would be concentrated at the surface and the land would go out of cultivation.

Some idea of what actually happened at Chakanwali may be gained from an inspection of Chart II. It shows how changes in areas of cultivated,

TABLE I.  
Area in acres of cultivated, banjar and ghairmunkin land in Kot Jan Bakhsh, Chakanwali and Paleh,  
from 1891-92 till 1921-22.

Village.	KOT JAN BAKHSH.				CHAKANWALI.				PALEH.				KALERWALA.			
	Cultivated area.			TOTAL.	Cultivated area.			TOTAL.	Cultivated area.			TOTAL.	Cultivated area.			TOTAL.
Year.	Cultivated area.	UNCULTIVATED.		TOTAL.	Cultivated area.	UNCULTIVATED.		TOTAL.	Cultivated area.	UNCULTIVATED.		TOTAL.	Cultivated area.	UNCULTIVATED.		TOTAL.
		Banjar.	Ghairmunkin.			Banjar.	Ghairmunkin.			Banjar.	Ghairmunkin.			Banjar.	Ghairmunkin.	
1891-92	800	431	35	1,266	358	494	39	891	676	511	76	1,263	959	1,119	30	2,108
1892-93	803	428	35	1,266	360	492	39	891	677	510	76	1,263	962	1,113	33	2,108
1893-94	806	425	35	1,266	360	492	39	891	674	508	81	1,263	965	1,110	33	2,108
1894-95	716	490	60	1,266	360	492	39	891	671	501	91	1,263	932	1,093	83	2,108
1895-96	716	489	61	1,266	360	492	39	891	671	501	91	1,263	933	1,092	83	2,108
1896-97	744	482	60	1,266	349	503	39	891	671	501	91	1,263	935	1,090	83	2,108
1897-98	751	454	61	1,266	354	498	39	891	621	551	91	1,263	960	1,065	83	2,108
1898-99	773	436	60	1,269	353	499	39	891	624	551	86	1,263	1,020	1,017	71	2,108
1899-1900	767	442	60	1,269	370	481	40	891	626	550	87	1,263	1,045	902	71	2,108
1900-01	772	437	60	1,269	497	354	40	891	745	432	86	1,263	1,238	786	82	2,108
1901-02	773	436	60	1,269	501	351	40	892	760	420	83	1,263	1,238	788	82	2,108
1902-03	816	392	61	1,269	507	344	40	891	760	420	83	1,263	1,192	832	84	2,108

1903-04	819	389	61	1,289	518	333	40	891	760	420	83	1,263	1,243	777	88	2,108
901-05	824	384	61	1,269	400	449	42	891	760	420	83	1,263	1,384	635	95	2,114
905-06	847	387	66	1,280	345	267	268	880	584	504	70	1,158	1,213	807	94	2,114
906-07	861	353	66	1,280	362	257	261	880	612	477	69	1,158	1,278	743	93	2,114
907-08	868	346	66	1,280	363	256	261	880	617	472	69	1,158	1,339	685	92	2,116
908-09	887	327	66	1,280	374	246	260	880	618	471	69	1,158	1,339	685	92	2,116
909-10	900	314	66	1,280	374	246	260	880	630	469	69	1,158	1,371	653	92	2,116
910-11	900	314	66	1,280	374	246	260	880	630	469	69	1,158	1,371	653	92	2,116
911-12	900	314	66	1,280	189	431	260	880	301	787	70	1,158	944	..	1,170	2,114
912-13	826	387	67	1,280	255	365	260	880	351	737	70	1,158	963	..	1,151	2,114
913-14	826	387	67	1,280	255	365	260	880	360	728	70	1,158	1,008	..	1,106	2,114
914-15	742	26	512	1,280	255	365	260	880	430	658	70	1,158	1,016	..	1,098	2,114
915-16	742	26	512	1,280	134	2	744	880	214	6	938	1,158	887	..	1,228	2,115
916-17	742	26	512	1,280	134	2	744	880	214	6	938	1,158	872	..	1,243	2,115
917-18	742	26	512	1,280	134	2	744	880	214	6	938	1,158	872	..	1,243	2,115
918-19	742	26	512	1,280	134	2	744	880	214	6	938	1,158	874	..	1,241	2,115
919-20	621	54	605	1,280	134	2	744	880	214	6	938	1,158	878	..	1,237	2,115
920-21	621	54	605	1,280	95	22	763	880	214	14	930	1,158	709	71	1,335	2,115
921-22	621	54	605	1,280	95	22	763	880	214	14	930	1,158	737	66	1,312	2,115



uncultivated (*banjar*) and unculturable (*ghairmumkin*) land occurred with the rise of the subsoil water, and also how at the same time the population of the village rapidly declined. The sudden rise in *ghairmumkin* in 1905 and again in 1916 at the expense of *banjar* is due to alterations made in revenue records in these years. This occurred after special settlements (*Jamabandis*), necessitated by pressing demands of the villagers, had been made.

It will be seen that the period 1891 to 1922 is divisible into four more or less distinct portions. During 1891 to 1899 the subsoil water was more than 10 feet below the surface. At this time out of a total area of 891 acres less than 40 acres were *ghairmumkin*, and about 350 acres were cultivated; the remaining land was left fallow and natural increase in population was noticeable. This was followed by a short spell of five prosperous years, when about 150 more acres were brought under cultivation out of the *banjar* land, due to a rise of subsoil water within 10 feet of the ground. With further rise in the water-table to 5 feet or less, a sudden change for the worse took place in 1905, when 120 acres went out of cultivation and were entered in revenue papers as *banjar*, but should have really been recorded as *ghairmumkin*. From 1905 to 1911 may be considered a period of struggle. The water-table had risen to about 4 feet and cultivation was carried out under difficult conditions. In spite of unusual prosperity in the first half of this decade the population dropped from 351 in 1901 to 181 in 1911. Since 1912, however, the land became so deteriorated that cultivation was more or less given up, except in small elevated patches of land where rice could be grown. The *ghairmumkin* area rapidly increased till over 740 out of 880 acres were declared as unculturable, and the population was reduced to 106. We have no detailed records of the agricultural conditions for 1922-26 but from all appearances the conditions, if anything, were continuously deteriorating.

### (3) ECONOMIC CONDITIONS.

There being no industrial or commercial concerns, the inhabitants of these villages are entirely dependent upon agricultural produce. The area of land under cultivation from year to year may roughly be taken to represent their economic condition, although extreme fluctuations in the price of agricultural products and the yield per acre, must necessarily have modified their gross income. The cultivated, *banjar*, *ghairmumkin* and the total acreage for each village from 1891-1922 are shown in Table I. Paleh seems to have suffered to about the same extent as Chakanwali. Kot Jan Bakhsh was not so unfortunate and did not suffer to the same degree as the other two villages, nor for as many years. Although, later, considerable portions of their land became *ghairmumkin*, yet the inhabitants of the village still had plenty of culturable land up to 1913. In fact from 1902 to 1913 they seemed to have enjoyed relative prosperity. Since 1914 some decline in the number of acres under cultivation is noticeable. It seems likely that this decline continued after 1922 till the land was taken

over by the Government. The agricultural conditions at Kalerwala were more or less comparable to those obtaining at Kot Jan Bakhsh.

There is little doubt, therefore, that Chakanwali and Paleh were subject to a severe and continuous economic strain for at least two decades before their land was acquired by the Government. Kot Jan Bakhsh and Kalerwala, though not so badly off, had also suffered considerable economic loss. Since the experimental farm was constituted, we are in a position to estimate the gross income of tenants at Kot Jan Bakhsh and Chakanwali. The figures given below (Table II) were computed by taking the share of the tenants for each product of the farm and estimating its cost according to the current market price. The total income thus obtained was divided by the number of families residing in the village.

TABLE II.  
*Showing average annual income of families.*

Year.						Kot Jan Bakhsh	Chakanwali.
1926-27	..	..	..	..	..	Rs. 230'28	Rs. 239'44
1927-28	..	..	..	..	..	„ 323'38	„ 273'33
1928-29	..	..	..	..	..	„ 579'26	„ 355'00
1929-30	..	..	..	..	..	„ 287'70	Not available.

It will be seen that since 1926-27 the income of the villagers has steadily increased; the decrease in 1929-30 is due to marked fall in the price of food grains. Thus the average wholesale price of wheat at Lyallpur (chief grain market) in May 1928 was Rs. 4-10-6, in May 1929, it came down to Rs. 4-3 and suffered a further decrease in 1930, when it was only Rs. 3-10 per *maund*.

#### IV. THE PEOPLE.

##### (1) POPULATION.

Kot Jan Bakhsh and Kalerwala may be considered as typical villages in this part of the country while Chakanwali, as it at present stands, is merely a collection of eight huts and hardly deserves the title of a village. The population of these villages, as determined by a house-to-house census conducted in January 1931, was 345, 486, and 32 respectively.

From Table III, Part I, which shows the variation in the size of population since the census of 1891, it is evident that instead of a natural increase, as seen during the first decade, these villages have suffered a progressive loss of population. The very sharp decline in population at Chakanwali since the 1921 census is due, mainly, to emigration of the villagers to the lands awarded to them by the Government. At Kalerwala the population has decreased but not to the

TABLE III.

## Part I.

*Census population of the three villages.*

Year.	Chakanwali.	Kot Jan Bakhsh.	Kalerwala.
1891 ..	279	461	455
1901 ..	351	576	688
1911 ..	181	485	606
1921 ..	106	386	854
1931 ..	32	471	486 (excluding Gajargola Station staff).

## Part II.

*Population of permanent residents determined by house-to-house visit.*

Year.	Chakanwali.	Kot Jan Bakhsh.	Kalerwala.
January 1929 ..	40	290	437
May 1929 ..	45	258	503
January 1931 ..	32	266	486

same extent as at Chakanwali. More or less the same conditions, as at Kalerwala, probably prevailed at Kot Jan Bakhsh, but the decline is not manifest in the census population of 1931, owing to the fact that it now includes farm employees. Census populations of Kot Jan Bakhsh are again misleading as they include a large number of persons belonging to a tribe of low class farm labourers (*changars*), who do not live in the village but have a settlement near the Gajargola distributary on the land entered in the Government papers as belonging to Kot Jan Bakhsh. Part II of Table III shows the changes in the population of tenants since 1929.

Besides these differences there is a frequent change of population of farm employees, some of whom live at Kot Jan Bakhsh while others reside at Paleh. In fact the main immigration and emigration during the period under observation was confined to the farm employees only (non-permanent residents). They came from Kharian Tahsil of Gujrat District and Jammu State and their malarial history was not known.

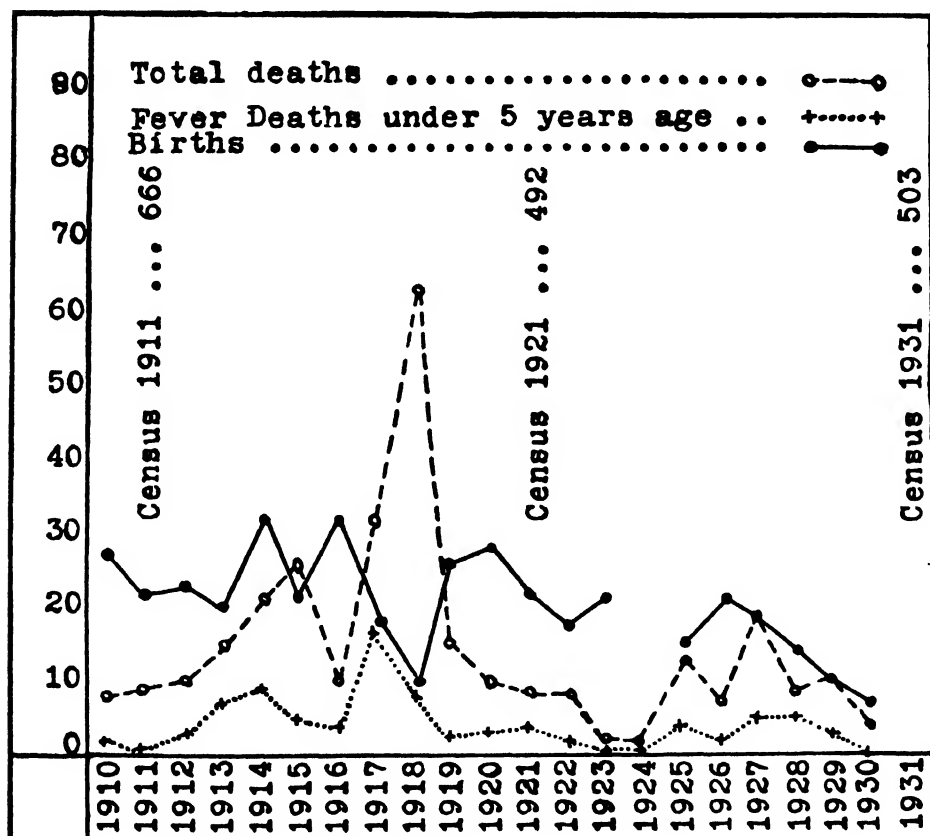
## (2) VITAL STATISTICS.

Registers for recording births and deaths are kept by the village watchman (*chaukidar*), who is generally illiterate and a part-time servant of the Government. The approximate age, religion, sex, occupation and cause of death are

recorded in these books. Chakanwali has neither a *chaukidar* nor a village headman (*lambardar*). Records for this village are kept by the *chaukidar* of Kot Jan Bakhsh. In Charts III and IV are shown the births, total deaths, and fever deaths under five years of age, for Kot Jan Bakhsh *cum* Chakanwali and for Kalerwala since 1910. Population for the census years are also shown.

CHART III.

Total deaths, fever deaths under 5 years of age and births each year from 1910 till 1930 at Kot Jan Bakhsh and Chakanwali



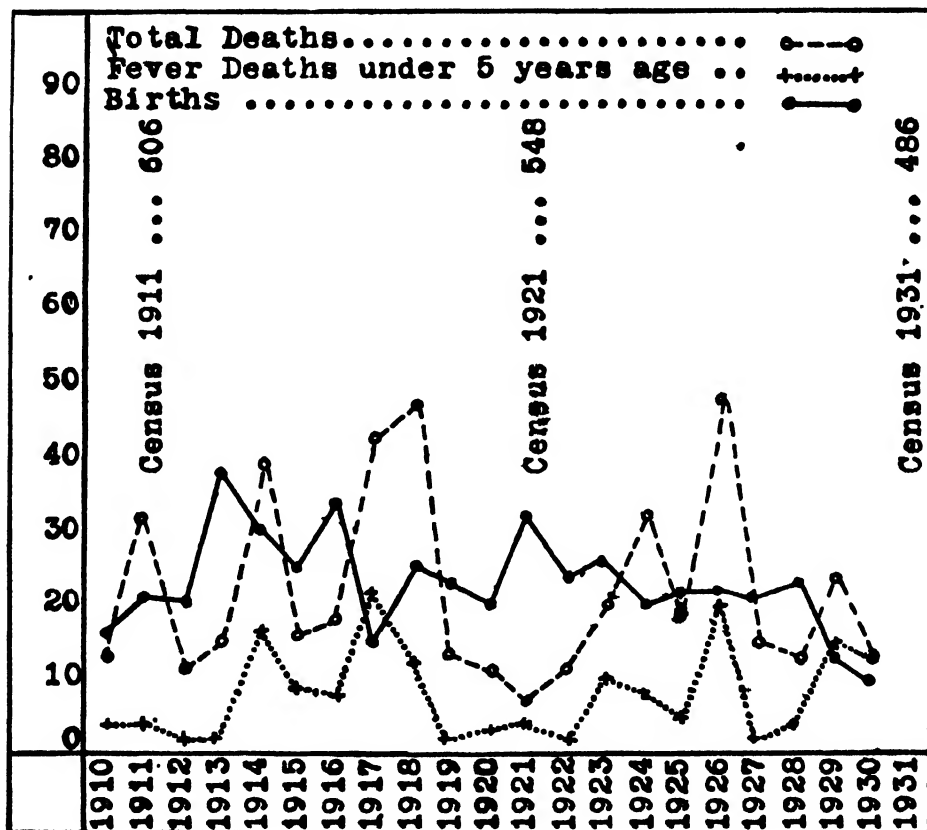
It will be noticed that at Kot Jan Bakhsh and Chakanwali births fluctuate more or less inversely as the total deaths. There is a steady decline in births after 1926 due to changes in constitution of the population, on account of the emigration of families of permanent residents and immigration of farm employees who generally left their families behind. Total deaths jump up in 1914-15, reaching a high level in 1917 and a well-marked climax in 1918 due to influenza. After this there is a general drop reaching a very low point in

1923-24, which is probably partly compensatory, and partly due to decreased population. Then there is again a slight rise, followed in 1930 by a drop below the level of 1910, but the death rate after 1926 is difficult to interpret for the reasons given above. Kalerwala births yield a curve closely resembling that of Kot Jan Bakhsh and Chakanwali except that :—

- (1) A low level was reached a year earlier, and
- (2) the subsequent rise started earlier, but was more gradual.

CHART IV.

Total deaths, fever deaths under 5 years of age and births each year from 1910 till 1930 at Kalerwala.



These differences seem to be correlated with the relatively high total mortality at Kalerwala in 1917 compared with that at the other villages. Total deaths in this village are subject to marked yearly fluctuation. The rise after the 1919-22 fall is well-marked. The high peak of 1926, which exceeds even the influenza peak, is due to plague. A small but distinct peak at Kalerwala in 1929 is due to malaria, as will be discussed later.

Total deaths, and deaths under 5 years, for the period under report are shown by months in Charts V and VI. Here also the Chakanwali data have been combined with those of Kot Jan Bakhsh. Nevertheless the universe of description is too small to allow us to draw any inferences. The comparison with the mortality reports at Kalerwala is rendered more difficult on account of differences in the constitution of the population in the two cases. However, the population according to 1931 census being nearly the same in both cases, one cannot fail to notice the decline in mortality, especially in autumnal deaths. This is more particularly amongst children at the farm villages, compared to that at Kalerwala.

CHART V.

Total deaths and deaths under 5 years of age in Chakanwali and Kot Jan Bakhsh combined, each month from November 1927 to January 1931.

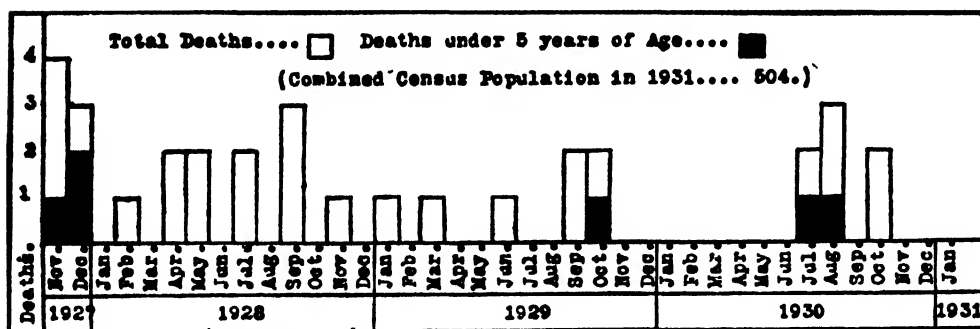


CHART VI.

Total deaths and deaths under 5 years of age in Kalerwala, each month from November 1927 to January 1931.

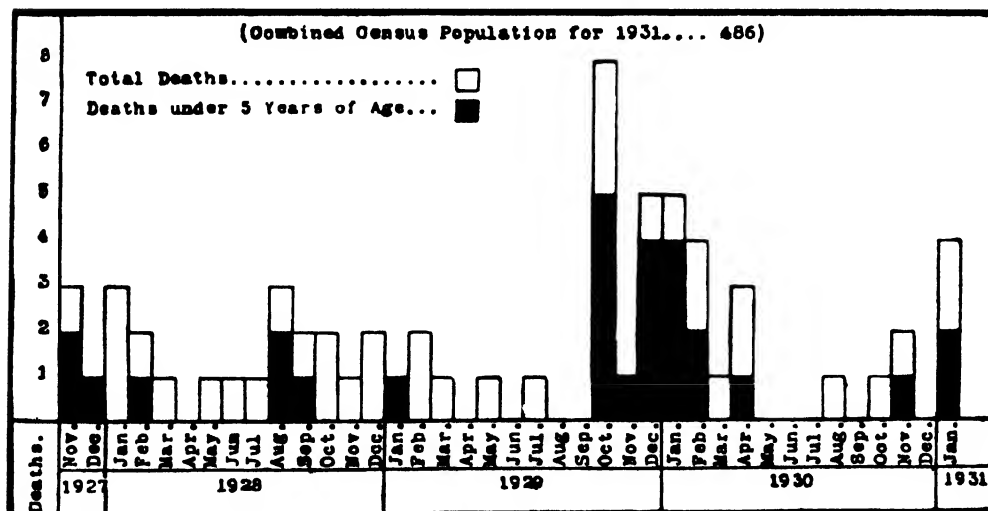


Table IV records census population for 1911 and 1921, total deaths, deaths under five years, as also number of deaths classified according to principal causes during 12 years (1910–1921) for a group of test villages, *viz.*, Kot Jan Bakhsh and Fatehpur and a group of control villages namely Mancher and Hazrat Kallianwala.

The test villages are situated in a badly water-logged tract, while the control ones are located outside the seriously affected area but are in the same rural circle. Since the villages were subject to an unusual decrease in population during this period, it has been considered advisable to base our calculation on the average population of two census years, *viz.*, 1911 and 1921. Four hundred and one deaths from all causes were recorded at the test villages during this period, as against 983 deaths at the control villages—the average crude death rates for the whole period being 46.54 and 39.48 respectively. Again, 325 fever deaths were registered at the former group as against 730 at the latter group—the crude fever death rate being 37.72 and 29.32. Unfortunately the age constitution in the two cases is not available. Perhaps the difference in the force of mortality would have been more marked if corrected death rates could be worked out. One would naturally expect a relatively smaller number of infants and old people, amongst whom death rate is highest, in the endemic areas. Another circumstance, which might further emphasize the unusual force of mortality in the test villages, is the fact that both Mancher and Hazrat Kallianwala, although taken as control villages, had not altogether escaped the effects of water-logging and had suffered a definite loss of population during the period under discussion.

It may also be observed that except for the influenza year (1918) the total deaths, as well as fever deaths, were more or less evenly distributed in the test villages, while in the control group besides that year, 1915, 1916 and 1917 were particularly bad. Total and fever deaths in 1917 were even more than those in 1918 and it would appear that, during these years, some unknown cause of death was operating in these villages, and that this was absent from the test villages.

### (3) HABITS AND CUSTOMS.

**Housing.**—An average family occupies a small house built of unbaked bricks (*kacha*). It consists of an open courtyard comprising about half the total area of the house site, with a cattle shed on one side, a combined living and bed-room, which is generally well lighted, and a dark chamber (*kothri*) where they keep their belongings. Some of the cattle sheds are open to wind and rain, others are dark and well sheltered. During summer, people sleep mostly outside in the open. They do not provide themselves with mosquito nets.

There are about three to four heads of cattle to a household, the total number of buffaloes and cows being a little more than the number of residents.

TABLE IV.

*The census population for 1911 and 1921, total deaths, deaths under five years as also number of deaths classified according to principal causes during, 1910 to 1921 for a group of test and control villages.*

Villages.	Cause of death.	1910	1911	1912	1913	1914	1915	1916	1917	1918	1919	1920	1921
Test villages Fatehpur and Kot Jan Baksh.	Total deaths	27	20	14	24	44	33	28	50	127	15	10	9
	Deaths under five	13	6	10	17	28	13	16	27	27	3	5	4
	Fevers	20	11	8	16	30	25	21	45	119	15	6	9
	Respiratory	..	3	..	..	..	1	2	..	2	..	2	..
	Euteric	..	..	..	..	..	..	..	..	..	..	..	..
	Others	7	6	6	8	14	7	5	5	6	..	2	..
Control villages Manohar and Hazrat Kallianwala.	Total deaths	83	54	55	54	66	91	93	150	128	72	61	76
	Deaths under five	43	23	29	35	32	16	67	90	27	43	29	45
	Fevers	63	41	42	39	35	24	77	131	122	57	43	56
	Respiratory	2	..	..	..	..	2	1	..	..	..	2	..
	Euteric	1	..	..	..	1	..	..	..	..	..	..	..
	Others	17	13	13	15	30	65	15	19	6	15	16	20

N.B.—Census population

	1911	1921
Test villages	..	822 614
Control villages	..	2,210 1,940







About 74 per cent of these are buffaloes, which being dark in colour are supposed to attract mosquitoes.

The houses are closely set together, and no screens are used for protection from mosquitoes. The average number of persons per house at Chakanwali, Kot Jan Bakhsh and Kalerwala is 5·4, 6·0 and 5·1 respectively.

**Dress.**—Due to economic and climatic conditions, people are scantily dressed. The summer wear of children may, in fact, consist of a loin-cloth only.

Thus the habits and the living conditions of the people expose them freely to mosquito bites. The villagers complain bitterly of the mosquitoes.

## V. THE ANOPHELINE FAUNA.

### (1) ADULT MOSQUITOES.

A uniform system of catching adult mosquitoes was adopted at each visit. Two hours in the morning were spent at each village; the search being confined to a group of selected houses and sheds. At Kot Jan Bakhsh a mosquito trap was used in addition. The records of catches by this method were kept separately.

The actual number of the *anopheles* of various species caught at each visit are shown in Tables V, VI and VII.

Several points in these records are of interest :—

(a) **Size of catches.**—They are slightly larger at Kot Jan Bakhsh than at Chakanwali but at both these villages they are considerably bigger than those at Kalerwala.

It seems that the number of mosquitoes caught was greater in 1929 and 1930 than during the earlier visits. However important this point may be, it is difficult to exclude errors due to the personal factor. A check is furnished by the mosquitoes trapped at Kot Jan Bakhsh. If these be compared with the number caught in this village in tubes, it would be evident that proportionately a much smaller number was caught by hand in 1928 than in 1929 and 1930, which suggests that the apparent increase during these years is possibly due to a more thorough search for the insects.

(b) **Seasonal incidence.**—Spring and autumn yielded the biggest catches, while during winter and mid-summer they were very small indeed. The decline in the number of mosquitoes with the advent of winter is very sudden. Similar features of variation in mosquito prevalence are also observable in non-water-logged areas in this region.

(c) **Species prevalence.**—As will be seen from Tables V to VII *A. stephensi* is the most predominant species at the farm, while at Kalerwala it is second only to *A. subpictus*. *A. culicifacies* comes next in all the three villages and then come *A. fuliginosus* and *A. pulcherrimus*. *A. maculipalpis*, *A. listoni* and *A. hyrcanus* have also been occasionally met with. From the point of view of malaria *A. stephensi* and *A. culicifacies* are decidedly the most important

species prevalent in these localities. They are not only the best known carriers of the malaria parasite in the Punjab and are present in very large numbers, but, more than any other species, they are constantly found throughout the year. No similar data are available for any non-water-logged area in this district and consequently we are unable to demonstrate any special features of the anopheline fauna of water-logged areas. However, applying the *chi*-square test to the total number of the various species of *anopheles* caught during the whole period of investigation, it is evident that the frequency distribution of

TABLE VI.

*The number of anopheles of various species caught at Kot Jan Bakhsh.*

Date of visit.	<i>A. culicifacies.</i>			<i>A. stephensi.</i>			<i>A. fuliginosus.</i>			<i>A. pulcherrimus.</i>		
	ADULTS.			ADULTS.			ADULTS.			ADULTS.		
	In houses.	In trap.	Larvæ.	In houses.	In trap.	Larvæ.	In houses.	In trap.	Larvæ.	In houses.	In trap.	Larvæ.
13-11-27 ..	65	0	11	13	0	4	11	0	3	8	0	0
7-12-27 ..	8	1	4	3	0	0	7	0	3	0	0	1
9-1-28 ..	9	0	0	1	0	0	1	0	0	0	0	0
10-3-28 ..	0	0	0	2	0	0	0	0	0	0	0	0
15-5-28 ..	0	0	0	7	0	4	1	0	6	0	0	0
12-6-28 ..	21	5	5	26	10	4	7	5	0	15	0	0
14-7-28 ..	13	5	5	15	13	0	9	0	0	2	0	0
20-8-28 ..	38	0	0	17	3	0	1	0	0	1	3	0
29-9-28 ..	13	0	16	39	4	30	12	0	4	31	0	14
25-10-28 ..	24	19	0	96	28	0	7	4	0	13	0	0
20-12-28 ..	1	0	0	9	0	2	0	0	0	0	0	0
22-1-29 ..	5	0	0	0	0	0	3	0	0	0	0	0
18-2-29 ..	2	0	0	3	0	0	0	0	0	0	0	0
29-3-29 ..	6	0	8	9	0	9	7	0	0	3	0	0
30-4-29 ..	18	N	0	27	N	0	6	N	0	0	N	0
30-5-29 ..	51	19	15	28	16	21	19	2	1	2	4	2
26-6-29 ..	53	8	8	145	15	4	9	2	1	9	0	0
26-7-29 ..	30	7	0	59	12	0	10	2	0	0	0	0
7-9-29 ..	18	0	0	63	3	0	7	0	0	0	0	0
15-10-29 ..	79	4	74	54	10	35	56	2	32	14	0	32
19-11-29 ..	78	4	4	76	6	14	28	3	7	4	0	2
19-12-29 ..	12	4	0	5	2	0	9	0	0	0	0	0
19-1-30 ..	95	0	9	12	0	26	13	0	19	2	0	0
15-2-30 ..	0	0	0	0	0	0	0	0	0	0	0	0
25-3-30 ..	1	0	4	8	0	1	10	0	9	5	0	6
24-4-30 ..	18	3	41	20	0	62	11	7	19	1	0	3
27-5-30 ..	16	0	28	119	14	31	90	11	33	29	0	5
22-6-30 ..	52	1	18	267	19	16	24	20	16	11	0	0
20-7-30 ..	24	3	24	64	17	22	10	0	14	1	0	0
23-8-30 ..	23	0	13	131	19	28	2	0	12	2	0	1
1-10-30 ..	13	0	8	28	0	29	8	0	11	11	0	7
2-11-30 ..	14	0	12	86	34	24	13	1	15	5	0	0
8-12-30 ..	20	0	1	36	0	18	6	0	0	2	0	0
26-1-31 ..	1	0	0	1	0	0	1	0	0	0	0	0

N = No record.

TABLE VI—contd.

Date of visit.	<i>A. subpictus.</i>			<i>A. maculipalpis.</i>			<i>A. listoni.</i>			<i>A. hyrcanus.</i>		
	ADULTS.			ADULTS.			ADULTS.			ADULTS.		
	In houses.	In trap.	Larvæ.	In houses.	In trap.	Larvæ.	In houses.	In trap.	Larvæ.	In houses.	In trap.	Larvæ.
13-11-27 ..	80	0	18	2	0	0	0	0	0	0	0	0
7-12-27 ..	4	0	4	0	0	0	1	0	0	0	0	0
9-1-28 ..	0	0	0	0	0	0	0	0	0	0	0	0
10-3-28 ..	2	0	0	0	0	0	0	0	0	0	0	0
15-5-28 ..	0	0	0	0	0	0	0	0	0	0	0	0
12-6-28 ..	8	0	2	1	0	0	0	0	0	0	0	0
11-7-28 ..	21	8	11	0	0	0	0	0	0	0	0	0
20-8-28 ..	20	7	0	0	0	0	2	0	0	0	0	0
29-9-28 ..	150	66	39	0	0	0	0	0	0	0	0	0
25-10-28 ..	86	19	0	2	0	0	1	0	0	2	0	0
20-12-28 ..	1	0	0	0	0	0	1	0	0	0	0	0
22-1-29 ..	0	0	0	0	0	0	1	0	0	0	0	0
16-2-29 ..	0	0	0	0	0	0	0	0	0	0	0	0
29-3-29 ..	0	0	0	0	0	0	0	0	0	0	0	0
30-4-29 ..	0	N	0	0	N	0	0	N	0	0	N	0
30-5-29 ..	0	0	0	0	0	0	2	0	0	0	0	0
26-6-29 ..	0	0	0	0	0	0	0	0	0	0	0	0
26-7-29 ..	0	0	0	1	0	0	0	0	0	0	0	0
7-9-29 ..	260	21	14	0	0	0	0	0	0	0	0	0
15-10-29 ..	113	19	52	1	0	0	2	0	0	1	0	0
19-11-29 ..	47	4	53	0	0	0	0	0	0	0	0	0
19-12-29 ..	2	0	0	0	0	0	0	0	0	0	0	0
19-1-30 ..	0	0	0	0	0	0	0	0	0	0	0	0
15-2-30 ..	0	0	0	0	0	0	0	0	0	1	0	0
25-3-30 ..	0	0	0	0	0	0	1	0	0	0	0	0
24-4-30 ..	0	0	0	0	0	0	0	0	0	0	0	0
27-5-30 ..	0	0	0	0	0	0	0	0	0	0	0	0
22-6-30 ..	0	0	0	1	0	0	0	0	0	0	0	0
20-7-30 ..	0	0	0	0	0	0	0	0	0	0	0	0
23-8-30 ..	187	17	26	0	0	0	0	0	0	0	0	0
1-10-30 ..	236	32	49	0	0	0	0	0	0	0	0	0
2-11-30 ..	156	49	19	1	0	0	1	0	0	0	0	0
8-12-30 ..	29	0	18	0	0	0	1	0	0	0	0	0
26-1-31 ..	0	0	0	0	0	0	7	0	0	0	0	0

N = No record.

the various species is identical at the two villages on the farm (*chi-square* = 0.26 P—more than 0.801253); but at Kalerwala it is materially different (*chi-square* = 90.3122876 P = 0.000000). Does this indicate difference in the environmental conditions or types of breeding ground? We shall have occasion to discuss this point later.

## (2) MOSQUITO LARVÆ.

At Kalerwala search for larvæ was made in all available collections of water, while at Kot Jan Bakhsh and Chakanwali a representative collection of

larvæ was obtained by searching for them, for at least two hours, in ponds, tanks, rice-fields, *kallar* plots, wells and drains of all sizes. Unfortunately no detailed records of the species of the larvæ caught in different types of breeding places were kept for the earlier visits, but later systematic records were kept. Tables V, VI and VII, which show the result of larval catches, suggest a general resemblance of larval and adult catches in seasonal distribution and species prevalence, except that in summer breeding was generally not quite so active as one would expect from the adult catches.

No dissections of female mosquitoes were carried out with a view to determining their age. The above facts, together with the presence of the male and the female mosquitoes in approximately the same proportion both in the adult and the larval catches, point, however, to the conclusion that the adult mosquito population in these villages was bred locally. In Table VIII a summary of relative adult mosquito prevalence, as well as breeding of *A. culicifacies* and *A. stephensi* at various seasons in the test and the control villages, has been given. Generally speaking both these species were less abundant at Kalerwala as compared with the farm. However, at both localities they were found in largest numbers in 1929 and in smallest numbers in 1928. Unlike other species these mosquitoes were breeding practically throughout the year, but like them their breeding was at its height in September, October and November. Considerable breeding took place in spring also, but in summer (June, July and August) the larval catches were generally poorer as compared with the adult catches and particularly so during the summer of 1929. Of the two species, *A. stephensi* bred freely in practically all available water collections, particularly in the drains carrying seepage water, while breeding of *A. culicifacies* was practically confined to the ponds and puddles. The latter fact probably explains the increasing predominance of the former species in the later period of investigation, when, due to reclamation, surface collections decreased and the drains increased. *A. fuliginosus* was also a drain breeder, but *A. pulcherrimus* preferred small puddles. Both *A. stephensi* and *A. culicifacies*, as well as *A. fuliginosus* and *A. subpictus*, bred well in *kallar* plots (pH 9.6). Fast running drains of seepage water (rate 2 feet per second) did not breed mosquitoes. Although the farm afforded good opportunities for experimenting with various anti-mosquito measures, we were not in a position to undertake this work for want of sufficient funds and staff.

## VI. INCIDENCE OF MALARIA.

### (1) MORBIDITY STATISTICS.

As has been previously noted, our direct observations depended on monthly visits and we had no agent of our own permanently residing on the farm. However, since the establishment by the Medical Department of a dispensary at Kot Jan Bakhsh, under the charge of a Sub-Assistant Surgeon, monthly







TABLE VIII.

*A summary of the relative adult mosquito prevalence as well as breeding of A. culicifacies and A. stephensi in various seasons in the test and the control villages.*

			1928		1929		1930	
			Farm area.	Kalerwala.	Farm area.	Kalerwala.	Farm area.	Kalerwala.
<i>A. culicifacies</i>	Spring	Larvæ	-	-	++	-	++	-
		Adult	±	-	+++	++	++	+
	Summer	Larvæ	+	-	-	++	++	++
		Adult	++	++	+++	++	+	+
	Autumn	Larvæ	++	++	++	+	+	+
		Adult	++	++	+++	++	+	+
	Winter	Larvæ	+	-	+	++	+	+
		Adult	+	+	+	+	-	+
	Spring	Larvæ	+	-	++	++	++	++
		Adult	+	+	++	+	++	+
	Summer	Larvæ	+	-	±	+	+	+
		Adult	++	++	+++	++	+++	++
<i>A. stephensi</i>	Spring	Larvæ	++	++	++	+	++	++
		Adult	++	++	++	+	++	++
	Summer	Larvæ	++	++	++	++	++	++
		Adult	++	++	++	++	++	++
	Autumn	Larvæ	++	++	++	+	+	+
		Adult	++	++	++	+	+	+
	Winter	Larvæ	+	+	+	-	+	+
		Adult	+	+	+	+	+	+

records of the total number of new patients attending the dispensary and those clinically diagnosed as malaria were obtained and are set forth in Table IX.

TABLE IX.

*A statement of the total number of new patients and malaria patients attending Kot Jan Bakhsh dispensary and the amount of quinine distributed.*

Number of visit.	Date of visit.	New patients.	Malaria patients	Amount of quinine consumed in ounces.
III ..	9- 1-28	130	36	..
IV ..	10- 3-28	360	52	..
V ..	15- 5-28	414	58	1½
VI ..	12- 6-28	532	59	2
VII ..	14- 7-28	508	58	2
VIII ..	20- 8-28	572	61	6
IX ..	20- 9-28	672	183	16½
X ..	25-10-28	340	37	2
XI ..	20-12-28	118	37	..
XII ..	22- 1-29	260	38	½
XIII ..	16- 2-29	230	56	..
XIV ..	29- 3-29	367	54	..
XV ..	30- 4-29	390	62	4
XVI ..	30- 5-29	499	50	4
XVII ..	26- 6-29	560	44	3·4
XVIII ..	26- 7-29	349	27	2
XIX ..	7- 9-29	460	45	2
XX ..	15-10-29	346	37	2
XXI ..	19-11-29	371	108	2½
XXII ..	19-12-29	272	69	½
XXIII ..	19- 1-30	281	38	½
XXIV ..	15- 2-30	262	36	½
XXV ..	25- 3-30	491	48	3
XXVI ..	24- 4-30	678	47	1½

These data could hardly be expected to yield any valuable information for the following reasons :—

- (1) Patients coming from Kot Jan Bakhsh or other villages on the farm were not shown separately from those coming from outside.
- (2) It took some time for the dispensary to become known in the surrounding villages and to become popular.
- (3) Diagnosis was based on clinical symptoms alone.

In order, however, to obtain comparatively more reliable data, a system of sickness cards was introduced in June 1929. The houses were numbered and each family was provided with a metal disc bearing a number corresponding to the house in which it lived. The Sub-Assistant Surgeon was provided with separate cards for each house in which age, sex, occupation, etc., of each member of the family were given. When attending the dispensary the metal disc had to be produced, thus enabling the medical officer to enter the diagnosis in the card against the name of the patient. At the end of the month these cards were collected and the data analysed as shown in Table X.

TABLE X.

Month.	Malaria fever	Accident	Other respiratory diseases	Diarrhoea, dysentery, etc.	Cholera.	Unclassified
July 1929 ..	0	1	1	4	0	15
August 1929 .	0	2	1	5	3	21
September 1929	10	0	1	2	0	12
October 1929			Data not collected			
November 1929	9	0	4	0	0	14
December 1929	8	0	3	1	0	9
January 1930 ..	3	0	4	5	0	7
February 1930	1	0	0	0	0	7
March 1930 ..	7	0	7	0	0	26
April 1930 ..	1	0	0	0	0	31

Unfortunately similar information for Kalerwala is not available, for, although similar cards were introduced in this village, very few persons could be induced to attend the dispensary at Kot Jan Bakhsh.

#### (2) SPLEEN CENSUS.

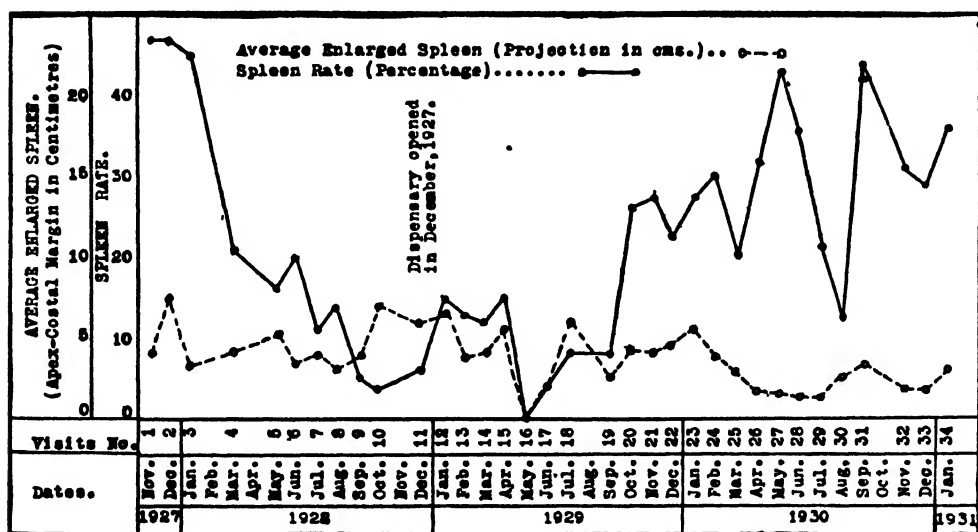
At Kot Jan Bakhsh, as nearly as possible, 25 children under 10 years of age and 25 adults were examined at each visit. At Chakanwali about 7 or 8 children, i.e., about half the total number of children in that village, were

examined. Since the opening of the school at Kalerwala on the 28th May, 1928, only school children were subjected to examination. After the first year's observations, only those permanently resident in these villages were examined. The spleen was always palpated with the patient in the standing posture and the enlargement of spleen was recorded in fingers as well as in centimetres and corrected according to the method of Christophers (*vide* Christophers *et al.*, 1928).

The spleen index and the average enlarged spleen are shown in Charts VII to X.

CHART VII.

Spleen rate and average enlarged spleen of children at Kot Jan Bakhsh and Chakanwali combined.



As the number of cases at Chakanwali was very small they have been considered together with those at Kot Jan Bakhsh.

**Spleen index.**— While the spleen index of children was approximately the same at Kalerwala and Kot Jan Bakhsh when the experiment began, it steadily declined in the latter village with the progress of the reclamation operations. In October 1928 it dropped down nearly to the base line and remained there till the following September, after which it ranged between 20 per cent and 30 per cent except for a spring and an autumnal rise. At Kalerwala on the other hand, the decline was more gradual and the minimum was not reached till July 1929. However, shortly afterwards it again began to rise till in November 1929 it

reached as high as 60 per cent. Since then it has maintained a high level between 50 per cent and 60 per cent or over, except for July when a spleen rate of only 27 per cent was recorded.

CHART VIII.

Spleen rate and average enlarged spleen of children at Kalerwala

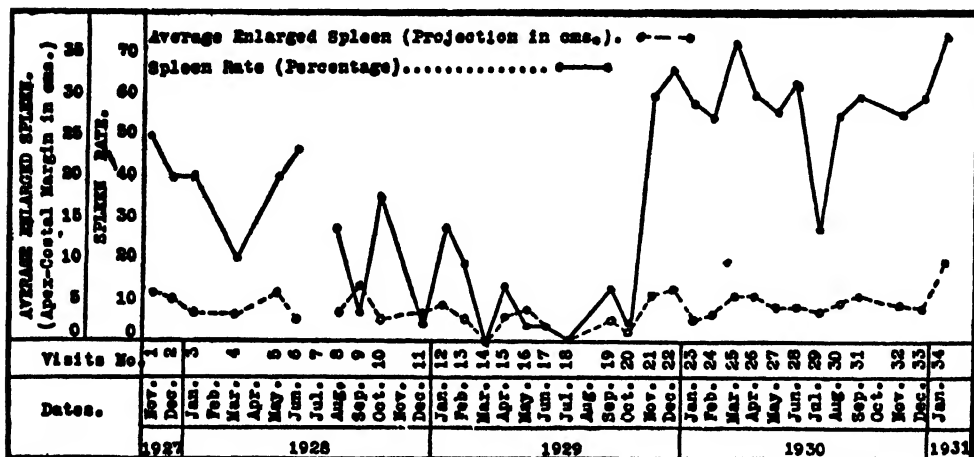
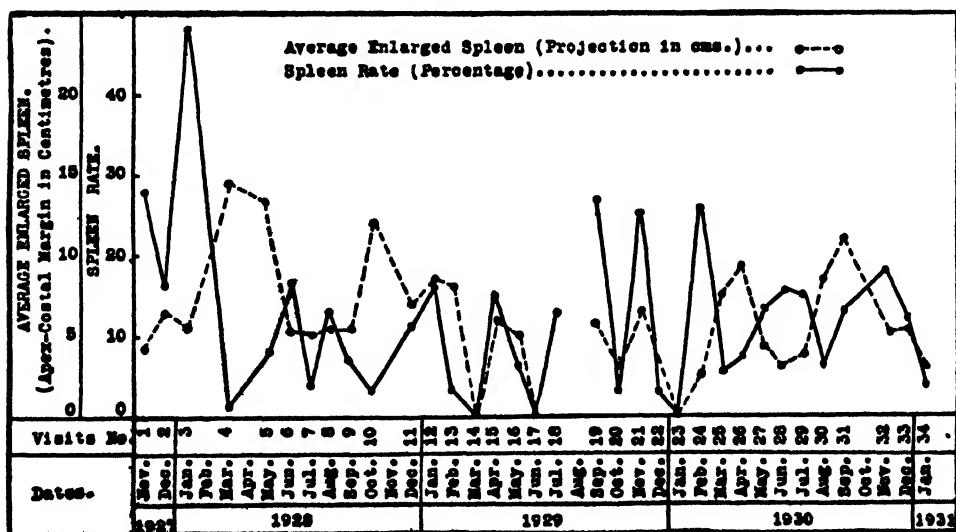


CHART IX.

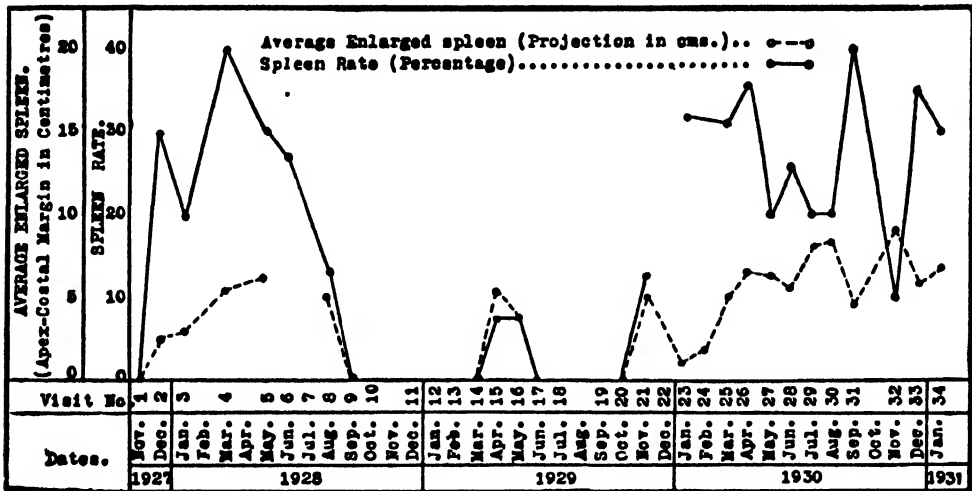
Spleen rate and average enlarged spleen of adults at Kot Jan Bakhsh and Chakanwali combined.



Except for a peak in January 1928 due to imported labour, the spleen index curve in adults at Kot Jan Bakhsh follows the same trend as that for children. At Kalerwala the highest adult spleen index, which was reached in March 1928, steadily declined to *nil* by the end of September. Practically no enlarged spleens were detected amongst the adults till October 1929, after which date the adult spleen index curve roughly followed the curve for children.

### CHART X.

Spleen rate and average enlarged spleen of adults at Kalerwala



From Chart XI which shows the frequency polygon of spleen enlargement in children at these villages for the whole period, it is evident that at Kot Jan Bakhsh and Chakanwali smaller degrees of spleen enlargement were more frequently found than at Kalerwala. This fact, taken in conjunction with a relatively low spleen rate at Kot Jan Bakhsh specially towards the close of the period of examination, seems to suggest that the recovery rate in this village was higher than at the control village.

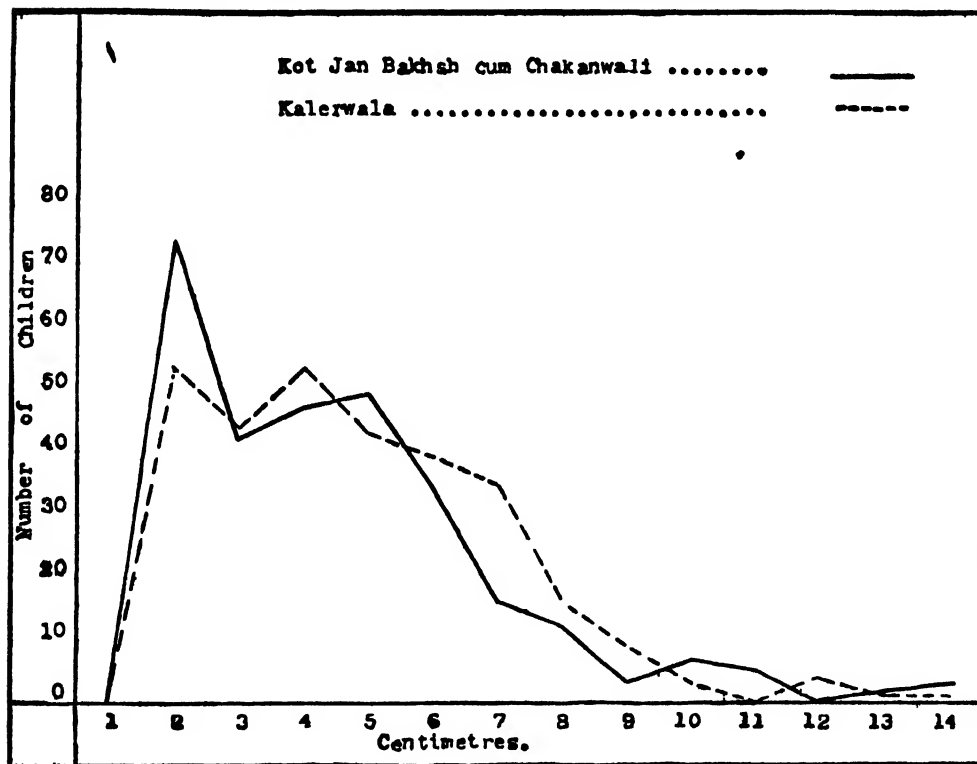
### (3) PARASITE RATE.

For blood examination the same random sample of children and adults was taken as for spleen census. Sinton's fowl cell suspension method of counting parasites (*vide* Christophers *et al.*, 1928) was used and both thick and thin films were prepared. The suspension used contained 12,500 cells per c.mm. and the search for parasites in all cases was continued till 500 fowl cells had been counted. At Kot Jan Bakhsh and Chakanwali (*see* Table XI) as many as 10 out of 32 children (31.2 per cent) examined on the first visit (November 1927) were found carrying malignant tertian parasites. Seven of these had

malignant tertian rings and three had crescents. Since then the parasite rate amongst children seems to have declined rapidly and remained low for the next twenty-two months. In October 1929 and thereafter, varying numbers of children with parasites in their blood were found at each visit, except in September 1930. This increase in parasite rate seems to be due to autumnal malaria of moderate severity followed by a fairly severe benign tertian infection in the spring of 1930, leaving behind some carriers of sexual forms. On the

CHART XI.

Frequency polygon of apex-umbilicus measurements of spleens of children  
(27th November, 1927 to 25th January, 1931).



other hand, no addition to parasite rate seems to have been contributed by autumnal malaria in 1930. The same position is suggested by the parasite rate for children at Kalerwala, except that there was little residual infection in 1927 and 1928. There was, however, some spring infection in the latter year and the parasite rate in 1930 was higher and more persistent. Amongst the adults, in both cases, the parasite rates exhibited the principal feature of the parasite rate in children, except that they were distinctly lower.

The average number of malaria parasites per c.mm. for each visit is recorded in Table XI. Generally speaking the average number of parasites

remained well below 400 per c.mm., except during the autumn of 1929 and the spring of 1930 when it went up to over 2,000. A comparative study of the two villages is not possible as the samples are very small.

TABLE XI.

*The malaria parasite infestation in Kot Jan Bakhsh cum Chakanwali and Kalerwala.*

*November 1927 to January 1931.*

Date of visit.		KOT JAN BAKHSH CUM CHAKANWALI.												
		CHILDREN.						ADULTS.						
		No. examined.	No. positive.	Average No. of parasites per c.mm.	M. T. R.*	B. T. R.*	M. T. G.*	B. T. G.*	No. examined.	No. positive.	Average No. of parasites per c.mm.	M. T. R.*	B. T. R.*	M. T. G.*
13-11-1927	32	10	1,600	7	0	3	0	32	1	625	1	0	0	0
7-12-1927	30	0	..	0	0	0	0	30	0	..	0	0	0	0
9-1-1928	20	0	..	0	0	0	0	28	0	..	0	0	0	0
10-3-1928	29	0	..	0	0	0	0	30	0	..	0	0	0	0
15-5-1928	23	0	..	0	0	0	0	23	0	..	0	0	0	0
12-6-1928	26	2	1,500	0	1	0	2	24	0	..	0	0	0	0
14-7-1928	36	0	..	0	0	0	0	28	0	..	0	0	0	0
20-8-1928	38	0	..	0	0	0	0	30	0	..	0	0	0	0
29-9-1928	37	0	..	0	0	0	0	28	0	..	0	0	0	0
25-10-1928	50	0	..	0	0	0	0	27	0	..	0	0	0	0
20-12-1928	36	0	..	0	0	0	0	28	0	..	0	0	0	0
22-1-1929	33	1	288	0	1	0	0	31	0	..	0	0	0	0
16-2-1929	38	0	..	0	0	0	0	31	0	..	0	0	0	0
29-3-1929	42	0	..	0	0	0	0	33	0	..	0	0	0	0
30-4-1929	36	1	425	1	0	0	0	34	0	..	0	0	0	0
30-5-1929	38	0	..	0	0	0	0	36	0	..	0	0	0	0
26-6-1929	38	2	1,812	2	0	0	0	30	0	..	0	0	0	0
26-7-1929	38	0	..	0	0	0	0	31	0	..	0	0	0	0
7-9-1929	31	0	..	0	0	0	0	30	0	..	0	0	0	0
15-10-1929	35	3	2,130	2	0	1	0	30	0	..	0	0	0	0
19-11-1929	29	4	1,225	2	0	2	0	27	3	2,400	0	0	2	1
19-12-1929	35	3	396	2	0	1	0	31	0	..	0	0	0	0
19-1-1930	35	5	384	2	1	2	1	24	0	..	0	0	0	0
15-2-1930	43	6	228	2	0	4	0	32	2	108	1	1	0	0
25-3-1930	35	5	198	1	3	0	1	34	0	..	0	0	0	0
24-4-1930	40	1	120	1	0	0	0	39	0	..	0	0	0	0
27-5-1930	35	3	1,650	1	0	1	1	31	2	132	1	0	0	1
22-6-1930	34	6	144	1	2	1	2	32	3	168	1	0	1	1
20-7-1930	33	3	147	0	2	0	1	24	0	..	0	0	0	0
23-8-1930	32	2	154	0	0	0	2	31	0	..	0	0	0	0
30-9-1930	32	0	..	0	0	0	0	30	0	..	0	0	0	0
1-11-1930	32	3	232	0	3	0	0	22	0	..	0	0	0	0
8-12-1930	24	1	220	0	0	1	0	25	0	..	0	0	0	0
26-1-1931	27	0	..	0	0	0	0	31	0	..	0	0	0	0

\* M. T. R. stands for malignant tertian trophozoites.

B. T. R. " benign " "

M. T. G. " malignant " gametocytes.

B. T. G. " benign " "



TABLE XI—contd.

KALERWALA.															
Date of visit.		CHILDREN.						ADULTS.							
		No. examined.	No. positive.	Average No. of parasites per c.mm.	M. T. R.*	B. T. R.*	M. T. G.*	B. T. G.*	No. examined.	No. positive.	Average No. of parasites per c.mm.	M. T. R.*	B. T. R.*	M. T. G.*	B. T. G.*
13-11-1927	..	10	0	0	0	0	0	0	10	0	0	0	0	0	0
7-12-1927	..	5	0	0	0	0	0	0	7	0	0	0	0	0	0
9-1-1928	..	5	0	0	0	0	0	0	10	0	0	0	0	0	0
10-3-1928	..	10	0	0	0	0	0	0	10	0	0	0	0	0	0
15-5-1928	..	10	0	0	0	0	0	0	10	0	0	0	0	0	0
12-6-1928	..	17	0	0	0	0	0	0	15	0	0	0	0	0	0
14-7-1928	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
20-8-1928	..	23	0	0	0	0	0	0	15	0	0	0	0	0	0
29-9-1928	..	15	0	0	0	0	0	0	12	0	0	0	0	0	0
25-10-1928	..	25	0	0	0	0	0	0	10	0	0	0	0	0	0
20-12-1928	..	25	0	0	0	0	0	0	17	0	0	0	0	0	0
22-1-1929	..	25	0	0	0	0	0	0	25	0	0	0	0	0	0
16-2-1929	..	17	0	0	0	0	0	0	13	0	0	0	0	0	0
29-3-1929	..	17	0	0	0	0	0	0	13	0	0	0	0	0	0
30-4-1929	..	25	0	0	0	0	0	0	25	0	0	0	0	0	0
30-5-1929	..	25	0	0	0	0	0	0	25	0	0	0	0	0	0
26-6-1929	..	25	0	0	0	0	0	0	11	0	0	0	0	0	0
26-7-1929	..	25	0	0	0	0	0	0	14	0	0	0	0	0	0
7-9-1929	..	31	3	214	1	1	1	0	10	0	0	0	0	0	0
15-10-1929	..	22	4	2,160	0	1	3	0	15	0	0	0	0	0	0
19-11-1929	..	20	7	12,580	0	0	6	1	16	2	12,146	2	0	0	0
19-12-1929	..	18	4	264	1	1	2	0	0	0	0	0	0	0	0
19-1-1930	..	25	5	235	5	0	0	0	25	0	0	0	0	0	0
15-2-1930	..	27	17	188	1	2	4	10	25	5	197	1	1	1	2
25-3-1930	..	25	3	200	0	0	0	3	16	2	216	0	1	0	1
24-4-1930	..	25	7	172	0	1	1	5	25	0	0	0	0	0	0
27-5-1930	..	20	1	132	0	0	0	1	25	0	0	0	0	0	0
22-6-1930	..	25	4	209	1	1	0	2	20	1	154	0	1	0	0
20-7-1930	..	22	3	154	0	2	0	1	16	0	0	0	0	0	0
23-8-1930	..	25	0	..	0	0	0	0	15	0	..	0	0	0	0
30-9-1930	..	20	2	220	1	0	0	1	25	1	198	0	0	0	1
1-11-1930	..	25	5	198	3	2	0	0	20	2	198	0	1	0	1
8-12-1930	..	25	3	136	0	0	1	2	23	0	..	0	0	0	0
26-1-1931	..	33	3	249	0	1	0	2	20	0	..	0	0	0	0

\* M. T. R. stands for malignant tertian trophozoites.

B. T. R. " benign " "

M. T. G. " malignant " gametocytes.

B. T. G. " benign " "

From October 1929 the imported farm labour was also examined. This population was subject to frequent changes in its number and individuals, as well as the localities from which it was drawn. However, as may be seen from Table XII, the labourers usually showed a higher gametocyte rate as compared with the farm residents and thus they constituted a reservoir of infection of malaria. A phenomenal rise in the gametocyte rate was observed amongst the

labour force residing at Paleh in June 1930, when as many as 22 persons out of 64 examined were found to carry the gametocytes of *P. vivax*. Almost all of them had suddenly gone down with fever in the previous month. This epidemic had also affected the permanent residents in the villages but to a much less extent, and it is probable that this batch of labourers was the focus of infection. Fortunately, however, these men left for their homes by the end of June.

TABLE XII.

*The malaria parasite infestation in Kot Jan Bakhsh labour force.*

Date of visit.	No. examined (adults).	No. with parasites.	B. T. R.	M. T. R.	B. T. G.	M. T. G.	Parasite rate, per cent.
16-10-29 ..	16	0	0	0	0	0	0
18-11-29 ..	30	2	0	0	1	1	6.7
18-12-29 ..	25	2	1	0	0	1	8.0
19- 1-30 ..	30	2	0	0	0	2	6.7
15- 2-30 ..	52	0	0	0	0	0	0
25- 3-30 ..	34	0	0	0	0	0	0
28- 4-30 ..	31	2	0	0	1	1	6.5
27- 5-30 ..	30	1	0	0	1	0	3.3
22- 6-30 ..	64	24	1	0	22	1	37.5
20- 7-30 ..	30	4	2	1	1	0	13.3
23- 8-30 ..	0	0	0	0	0	0	0
1-10-30 ..	0	0	0	0	0	0	0
2-11-30 ..	0	0	0	0	0	0	0
8-12-30 ..	0	0	0	0	0	0	0
26- 1-31 ..	28	0	0	0	0	0	0

Examination of *changars* living at *Kuli Changran* (near the Gajargola distributary) was also carried out from October 1929 (see Table XIII). These people seem to be subject to malaria infection to a greater extent than the residents of Kot Jan Bakhsh and Chakanwali.

TABLE XIII.

*The malaria parasite infestation in Kuli Changran.*

Date of visit.	Number examined.	No. with parasites.	B. T. R.	M. T. R.	B. T. G.	M. T. G.	Parasite rate, per cent.
18-11-29 ..	Children 15	0	0	0	0	0	0
	Adults 10	1	0	0	1	0	10.0
18-12-29 ..	Children 12	2	1*	0	1	1*	16.7
	Adults 6	0	0	0	0	0	0
19- 1-30 ..	Children 20	0	0	0	0	0	0
	Adults 19	0	0	0	0	0	0
15- 2-30 ..	Children 29	7	1	0	6	0	24.1
	Adults 15	0	0	0	0	0	0
25- 3-30 ..	Children 28	11	0	3	6	2	39.3
	Adults 7	0	0	0	0	0	0
28- 4-30 ..	Children 21	0	0	0	0	0	0
	Adults 4	0	0	0	0	0	0
27- 5-30 ..	Children 20	2	0	1	1	0	10.0
	Adults 15	0	0	0	0	0	0
22- 6-30 ..	Children 25	3	1	1	1	0	12.0
	Adults 20	1	1	0	0	0	5.0
20-7-30 ..	Children 25	4	1	1	1	1	16.0
	Adults 15	1	0	0	1	0	0
23- 8-30 ..	Children 25	1	0	0	1	0	4.0
	Adults 11	0	0	0	0	0	0
1-10-30 ..	Children 25	4	1	1	2	0	16.0
	Adults 15	0	0	0	0	0	0
2-11-30 ..	Children 25	9	2*	2*	6	0	36.0
	Adults 10	0	0	0	0	0	0
8-12-30 ..	Children 25	6	2	1	3	0	24.0
	Adults 10	0	0	0	0	0	0
6- 1-31 ..	Children 30	8	2*	0	8*	0	26.7
	Adults 15	0	0	0	0	0	0

\* Represent double infection.

**VII. SUMMARY OF OBSERVATIONS.**

- (1) An elaborate system of canal irrigation has been opened up for the cultivation of vast tracts of land in the Punjab, which were lying barren for want of water. This has added immensely to the prosperity of the Province.
- (2) In restricted portions of the irrigated areas a gradual rise of water-table due to percolation from the canals has brought about conditions of water-logging, and has caused the alkaline salts of the soil to concentrate near the surface. Lands in the affected areas have become unfit for cultivation, the people have suffered great economic loss, their health has been seriously affected, mainly through chronic malaria, and many homes have been deserted.
- (3) The Government in 1926 acquired by exchange 3,465 acres of land in one of the worst water-logged tracts along the Lower Chenab Canal. This tract was used for experimental reclamation and was constituted into what is known as the Chakanwali Reclamation Farm.
- (4) Reclamation operations consisted mainly of agricultural adjustments and an elaborate system of open seepage drains, which carried away the surface water and maintained a continuous movement of the subsoil stream.
- (5) Commencing in November 1927, monthly visits to the farm villages Kot Jan Bakhsh and Chakanwali and also to Kalerwala—a control village situated in the water-logged tract outside the farm—were made for over three years, with a view to investigate the health conditions of the villagers, as affected by water-logging and reclamation operations.
- (6) The farm is situated in the *Rechna Doaba* between the Ravi and the Chenab rivers. It is subject to extreme weather conditions, *viz.*, excessive heat in summer and excessive cold during winter.
- (7) It lies in the angle between the Lower Chenab Canal, which carries a continuous stream of large volume of water at a high level, and one of its distributaries—the Gajargola distributary. These cause a considerable amount of seepage, and, together with the railway line, occasion obstruction to the natural flow of surface water towards the river.
- (8) Since the opening of the canal in 1894, the water-table which was formerly as deep as 25 feet began to rise. For a short period between 1900 and 1904, when the subsoil water level was about 10 feet, cultivation was considerably improved, but with a further rise of the subsoil water level crops began to fail and field after field went out of cultivation.

- (9) At the time the farm was taken over by the Government more than four-fifths of the area was unculturable, large expanses of permanent water collections were found near the villages, people were suffering from chronic malaria and acute economic stress.
- (10) Since 1926 large areas have been reclaimed and the economic condition of the tenants, who had remained behind, has greatly improved.
- (11) From the time the land was damaged by water-logging, the population of these villages has suffered progressive loss due to emigration. This was much more marked at Chakanwali than at Kot Jan Bakhsh and Kalerwala. In fact the population of Chakanwali was reduced from 351 in 1901 to 40 in 1926 and Paleh, another village on the farm, became entirely de-populated.
- (12) Census population of Kot Jan Bakhsh is, however, complicated by two circumstances. It includes a colony of low caste labourers (*changars*) who live on the other side of the Gajargola distributary, more than one mile and a half from the village proper. Since 1926 it also includes a floating population of farm employees who are imported from outside, from time to time, and many of whom leave for their homes when their job is finished. At Kalerwala, on the other hand, there were relatively few changes.
- (13) Records of births and deaths are kept by illiterate village watchmen and entries as regards the cause of death are, therefore, very unreliable. However, during the period of water-logging, these villages were not only subject to a definitely higher degree of mortality than some other villages situated in the same neighbourhood outside the affected areas, but their death rate did not show the same yearly fluctuations as that of the latter group.
- (14) The habits and customs of the villagers are such as would fully expose them to the bites of mosquitoes especially at night. Their food consists mostly of cereal grains and vegetables, wheat being the staple article of diet.
- (15) Mosquitoes were found to breed at the farm in abundance, and plenty of adults could be caught especially during the spring and the autumn. At Kalerwala the adult catches were generally smaller and breeding was less abundant.

Of the malaria-carrying species *A. stephensi* and *A. culicifacies* were the most predominant. They were found in more or less large numbers throughout the year. Low velocity seepage drains formed the main breeding grounds for *A. stephensi*, though they were also frequently found in the ponds. *A. culicifacies* was almost wholly restricted to stationary water collections especially the small pools and puddles after the rains. High salinity of the soil and its alkalinity did not discourage the breeding of these species nor that of *A. fuliginosus* and *A. subpictus*. *A. fuliginosus*, though a poor

carrier of malaria, was found in very large numbers and probably played a definite rôle in the spread of infection. Other species of anopheline found were *A. subpictus*, *A. pulcherrimus*, *A. listoni*, *A. maculipalpis* and *A. hyrcanus*. With the progress of reclamation, there was, if anything, an increase in mosquito population. However, this increase was probably more apparent than real and was to a large extent due to personal factors in mosquito collection.

- (15) Morbidity statistics are not sufficiently satisfactory to form a basis for discussion. Reliable and detailed records of sickness amongst the permanent residents at the farm villages are available from July 1929 to April 1930, but corresponding figures for Kalerwala are not comparable, as residents of this village could not be induced to attend the dispensary at Kot Jan Bakhsh.
- (16) The spleen rates were high both at the test and the control villages when the investigation began. At the farm it rapidly declined and remained below 15 per cent from July 1928 to September 1929. After this, it went up and ranged between 20 to 40 per cent for the rest of the period. At Kalerwala the decline was more gradual and spleen rate below 15 per cent was registered only for a short period, *viz.*, during February to October 1929. Subsequently it remained in the vicinity of 60 per cent.

The average enlarged spleen was also higher at Kalerwala than at the farm villages during the latter part of the investigation.

- (17) Parasite rates followed the spleen indices at both the test and the control villages, except that at Kalerwala parasite rate was low in autumn 1927 and consequently residual infection in the beginning of 1928 was not high. There was, however, a small spring epidemic during this year.

### VIII. DISCUSSION OF RESULTS.

It is now possible to examine how the above-recorded observations, singly and in relation to each other, stand with respect to the factors concerned in the transmission, infection and recovery from malaria, in these localities, *i.e.*, the relative part played by the mosquito, the parasite and the human factors. An attempt must be made to ascertain, if possible, how far conditions of water-logging and the reclamation work have affected the malaria problem.

#### (1) THE MOSQUITO FACTOR.

Its geographical position and low altitude place the farm in a zone which is well known for its abundance of malaria-carrying anophelines. The water surface, exposed in the drains and the ponds, is large and suitable for mosquito breeding as has been proved by actual larval catches. The high saline content and alkalinity of the soil evidently do not inhibit the breeding of the mosquito

species found, viz., *A. culicifacies*, *A. stephensi*, *A. fuliginosus*, *A. pulcherrimus* and *A. subpictus*.

The subsoil water level is high and this, together with the alkalinity of the upper layers of the soil, helps to maintain an extensive water surface, after the rains. This water occurs mainly as small puddles and lasts for periods long enough to allow mosquitoes to breed.

Being situated at a greater distance from the canal, the subsoil water level at Kalerwala is comparatively low and the actual water surface is much smaller than at the farm. These facts evidently account for the lower adult mosquito and larval catches at this village as compared with those at the other two villages.

Of the prevalent species, *A. stephensi*, by reason of its being an excellent carrier of malaria parasite in this country and its constant presence in very large numbers throughout the year, must necessarily be considered as potentially the most dangerous mosquito. It was found breeding in seepage drains, ponds, and puddles, in fact in every place where clean water was to be found. *A. culicifacies* was also quite common throughout the year and being the best known carrier was almost equally dangerous. It was found breeding chiefly in ponds. Of other species *A. listoni*, although a very efficient carrier, was not found in abundance and may perhaps be considered of less importance than *A. fuliginosus*, which, though a poor carrier, was found in very large numbers. *A. maculipalpis* was found breeding in pools, but, being a doubtful carrier and met with in very small numbers, was not likely to be of much importance.

Five hundred and twenty-nine *A. culicifacies*, 430 *A. stephensi* and 237 *A. fuliginosus* were dissected for the presence of the parasite at different visits. None was found infected. Much significance cannot be attached to this experience as the number of dissections was relatively small.

The cattle are not very numerous; they are kept at night in sheds inside the dwelling houses scattered throughout the village. The most dangerous prevalent species, viz., *A. culicifacies* and *A. stephensi*, are not cattle feeders, hence conditions for zoophilism to act are not at all favourable. However, the cattle, and especially the buffaloes, may attract *A. fuliginosus* and *A. subpictus* and lessen the annoyance of mosquito bites to man.

There is no jungle or belt of dense forest nearby, which might afford protection for the mosquitoes. The houses are, however, made of mud which keeps them cool in summer. Dark chambers and cattle sheds are abundant, hence the mosquitoes are almost entirely house dwellers.

We have thus seen that locally bred mosquitoes, of species which are very good malaria carriers, are found in abundance inside the houses throughout the year, and that cattle are not likely to afford protection to man against their bites. From this, however, it cannot be assumed that they are necessarily dangerous transmitters of malaria. For this assumption it has to be shown that certain meteorological conditions are present. These factors were brought into prominence by Gill (1921), who showed that they were requisite for the

development of the exogenous cycle of the parasite and for the sustenance of life of the insect for a sufficiently long period to allow it to become infective and remain so for some time.

For long life the mosquito requires a high degree of humidity, but the temperature range may be fairly wide. Although we do not know the exact temperature limits, they have been found hibernating in the severest winter and are alive even at a temperature as high as 40°C. (104°F.). Under these conditions of temperature *A. culicifacies* and *A. stephensi*, the species with which we are mostly concerned here, not only live but exhibit great activity.

In the summer months the temperature during the day, as recorded by the thermographs placed in the open in Stevenson's screens, may mount up as high or higher than 110°F. or even 120°F. The minimum daily temperature, however, remains below 100°F. and, taking into consideration the opportunities that mosquitoes have of hiding in dark corners in the houses, we might say that the temperature factor by itself never becomes inimical to mosquito life. In fact relatively more adult *A. stephensi* and *A. culicifacies* were caught during the middle of summer than one would expect from the rate of their breeding. This suggests that the adults could manage to live for a long time even in this season.

The humidity factor was perhaps not quite so favourable. For the greater part of the year and specially during March, April and May and also in September, October and November, the minimum daily humidity was below 45 per cent which is the critical point for the maintenance of mosquito life. On the other hand, the daily maximum humidity remained well above 70 per cent almost throughout the period of observation, the only exception being during March, April and May 1929 when the humidity figures were very low. We may, therefore, assume that the climatic factors in 1928 and 1930 were not altogether unfavourable for long life of the mosquitoes during the winter, late summer and autumn. This, however, was not the case in the spring of 1929 according to the available records. Even so, adults of *A. stephensi* and *A. culicifacies* were caught in large numbers during this period, and obviously the insects were able to find suitable resting places in the cooler parts of the houses. According to Jancso the optimum range of temperature for the completion of the exogenous cycle of *P. vivax* and *P. falciparum* is 68°F. to 86°F. According to the same authority, the minimum temperature, at which the cycle can be completed, is 60.8°F. The humidity, as shown by Gill, is not important so long as it is high enough to support mosquito life. The conditions for the completion of the exogenous cycle of the parasite are, therefore, satisfied throughout the period of investigation except during the winter months when the possibility of the parasite completing its cycle may be ruled out. Moreover other factors such as (a) the habits of mosquitoes during the winter months, (b) the better covering worn by the people at night against cold, affording protection from mosquito bites, and (c) the sudden drop in the



percentage of male crescents in the circulating blood (Lal, 1925), reduce the chances of human infection during this period to the minimum.

## 2. THE HUMAN FACTOR.

(a) **As reservoir host of the parasite.**—Of 32 children examined at Kot Jan Bakhsh and Chakanwali by Colonel Gill in August 1922, 3 were found carrying malaria parasites in their blood, one out of whom had gametocytes of *P. vivax*.

The number of gametocyte carriers found during the course of this investigation is given in Table XIV.

It would appear that the permanent population of these villages had recovered from malarial infestation to such an extent that gametocyte carriers could hardly be discovered till October 1929. Since then carriers, though small in numbers, have constantly been met with. In the earlier period of the investigation one of the factors against the spread of infections was, perhaps, an insufficiency of gametocyte carriers. Since the autumn of 1929 considering the presence in large numbers of good malaria-carrying species of mosquitoes, the number of gametocyte carriers may be considered adequate for the spread of infection.

The labour employed at the farm, being drawn mostly from malaria-ridden localities, constituted an additional source of infection at Kot Jan Bakhsh. It was, however, a very uncertain factor because the labour force was liable to great variation both in regard to its number and composition.

In interpreting these results the question naturally arises as regards the method of sampling and the sampling errors. All permanent residents, who could be induced to subject themselves to the examination, were examined and no selection was consciously made except that mostly school children were examined at Kalerwala. The question of random sampling and the adequacy of the samples will be discussed later.

(b) **As regards susceptibility to infection.**—The recorded data with respect to the previous malaria history of these people are very meagre. It is unfortunate that our regular observations commenced a year after the reclamation operations had begun. But from what we know, it appears that this area was badly affected with chronic malaria, repeated attacks were common and recovery was very slow. Economic stress was acute and accounted for considerable emigration. The death rate, especially from fevers, was higher than that in the surrounding country outside the badly water-logged tract. It would thus give us a picture of an endemic centre with a population possessing a high degree of immunity to malaria. Furthermore the landholders emigrated from the villages of Kot Jan Bakhsh and Chakanwali in 1926, leaving behind the lower strata of society which are commonly believed to be more liable to infectious diseases. During the earlier part of our observations in both communities the spleen rate was high among the children and moderate in adults. However, at the farm the fall in spleen rate was rapid while at Kalerwala it was more

TABLE XIV.

Date of visit.	KOT JAN BAKSH cum CHAKANWALI.				IMMIGRANTS.				KULI CHANGHAN.				KALERWALA.			
	No. of adults and children examined.	B. T. G.*	M. T. G.†	Gametocyte rate (percentage).	No. of adults and children examined.	B. T. G.*	M. T. G.†	Gametocyte rate (percentage).	No. of adults and children examined.	B. T. G.*	M. T. G.†	Gametocyte rate (percentage).	No. of adults and children examined.	B. T. G.*	M. T. G.†	Gametocyte rate (percentage).
13th Nov., 1927	64	0	3	4.7	..	..	..	..	..	..	..	..	20	0	0	0.0
12th June, 1928	50	2	0	4.0	..	..	..	..	..	..	..	..	32	0	0	0.0
7th Sept., 1929	61	0	0	0.0	..	..	..	..	..	..	..	..	41	0	1	2.4
15th Oct., 1929	65	0	1	1.5	16	0	0	0.0	..	..	..	..	37	0	3	8.1
10th Nov., 1929	56	1	4	8.9	30	1	1	6.7	25	1	0	4.0	36	1	6	19.4
19th Dec., 1929	66	0	1	1.5	25	1	0	4.0	18	1	1	11.1	18	0	2	11.1
19th Jan., 1930	59	1	2	5.1	30	0	2	6.7	39	0	0	0.0	50	0	0	0.0
15th Feb., 1930	75	0	4	5.3	52	0	0	0.0	44	6	0	13.6	52	12	5	32.7
25th Mar., 1930	69	1	0	1.4	34	0	0	0.0	35	6	2	22.9	41	4	0	9.8
24th April, 1930	79	0	0	0.0	31	1	1	6.5	25	0	0	0.0	50	5	1	12.0
27th May, 1930	66	2	1	4.5	30	1	0	3.3	35	1	0	2.9	45	1	0	2.2
22nd June, 1930	66	3	2	7.6	64	22	13	5.9	45	1	0	2.2	45	2	0	4.4
20th July, 1930	57	1	0	1.8	30	1	0	3.3	40	2	1	7.5	38	1	0	2.6
23rd Aug., 1930	63	2	0	3.2	..	..	..	..	36	1	0	2.8	40	0	0	0.0
30th Sept., 1930	62	0	0	0.0	..	..	..	..	40	2	0	5.0	45	2	0	4.4
1st Nov., 1930	54	3	0	5.6	..	..	..	..	35	6	0	17.1	45	1	0	2.2
8th Dec., 1930	49	0	1	2.0	..	..	..	..	35	3	0	9.6	48	2	1	6.3
26th Jan., 1931	58	0	0	0.0	28	0	0	0.0	45	8	0	17.8	53	2	0	3.8

\*B. T. G. = Gametocytes of *P. vivax*.†M. T. G. = Gametocytes of *P. falciparum*.

gradual. It may thus be assumed that, on account of almost complete absence of fresh infections in 1928, both the communities had pretty well recovered from malarial infestation and had consequently lost a great deal of their immunity before autumn 1929. Balance between infection and immunity being thus upset a sharp autumnal epidemic, followed by a benign tertian spring epidemic, occurred at both places in 1929-30.

(c) **As regards recuperative power.**—The material for discussing this problem may be divided into two parts namely :—

- (1) Direct evidence consisting of morbidity and mortality rates, and
- (2) indirect evidence consisting of spleen and parasite rates.

As we have already seen, the villages on the farm were subject to a more constant and much severer force of mortality than the control villages situated outside the water-logged area. In the latter part of our investigation there is a decline in the mortality rate at the farm which seems to be greater than that at Kalerwala. This is particularly marked in autumnal deaths amongst children. The deaths recorded at Kot Jan Bakhsh include those amongst the employees and the *changars*, that is in a combined population of 471 as against 486 at Kalerwala. The changes in the constitution of the farm population, however, introduce a complicating factor which cannot be easily analysed. Moreover the period of observation and the universe of description are so small, that we are unable to draw any definite conclusions.

The morbidity statistics, as has been explained before, are of still less value. We have, therefore, to depend chiefly on the indirect evidence. In interpreting these data the question of goodness of sample and sampling error naturally arises. Up to the end of 1928, examination had not been strictly confined to the tenants at Kot Jan Bakhsh and in fact the sharp rise in the spleen rate of adults in January of that year was due to the Pathan labour employed at the time. Since, however, the senior author took charge of the investigation (January 1929) all but the permanent residents (tenants) were excluded from examination.

All available persons were examined and no selection was consciously made except that school children were mostly examined at Kalerwala. We are unaware of any other circumstances which might have affected random sampling. In fact a statistical study of the frequency distribution of individuals with enlarged spleen having been examined once, twice and thrice, etc., shows that sampling was fair and similar in the test and the control villages.

As to the adequacy of the samples of permanent residents, we have the following statement (Table XV). In the case of imported labour almost all persons were examined.

It is obvious that a fair sample was drawn in each case, except in the case of Kalerwala adults where the average sample was only about 5 per cent of the population. While a slight difference in the parasite rate in favour of Kot Jan Bakhsh and Chakanwali may be noticed in Table XI, this by itself is not significant. The spleen rates, however, definitely suggest the greater recuperative power of Kot Jan Bakhsh and Chakanwali population, as compared with the community at Kalerwala. In order to investigate this point further, histories with regard to spleen enlargement of different individuals were traced for the whole period of observation. The results obtained are tabulated below (Table XVI).



Singh it produced some of his ablest generals, as well as bravest followers. We may, therefore, assume that before the advent of the canal this neighbourhood was peopled by healthy virile cultivators. In the beginning of this century when the water-logging conditions were established and economic condition became low the cultivators began to desert their homes in search of livelihood elsewhere. The less adventurous of them, or those whose vested interests kept them back, fell an easy prey to malaria. With their lowered vitality they failed to recover fully. A constantly high death rate further pointed towards the insalubrity of the area. This in fact was the picture painted by Colonel Gill (1922).

With the progress of reclamation operations, ponds, depressions and other surface collections were drained but in their place an elaborate system of surface drains came into being. Although the actual subsoil water level was not materially lowered, the movements of the subsoil stream were greatly accelerated thus preventing accumulation of surface water after the rains and causing more rapid drying up of the upper layers of the soil. Large areas of land were thus made available for cultivation giving plenty of remunerative occupation to the tenants. Extensive permanent breeding places for *A. culicifacies* were obliterated but many field drains provided suitable breeding ground for *A. stephensi* and *A. fuliginosus*. In some of the fast running drains which kept clean, mosquito larvæ were not found. The effects of drainage on humidity were not clearly marked. Drainage did not reduce the actual number of dangerous malaria-carrying species on the farm nor did it cause their life to be shortened.

On the other hand, we find that at the farm there was a more rapid recovery from malaria than at Kalerwala during the earlier part of 1928, as evidenced by a relatively steeper decline in the spleen rate. Absence of epidemic malaria in 1928 at both the control and the test villages gave some relief to the local population. This, however, was not confined to the group of villages under observation, but was of more general occurrence in this neighbourhood. This is evidenced by the fact that the epidemic figure\* for both Akalgarh and Hafizabad rural circles was only 0.5. What circumstances were responsible for the unusual salubrity of this year we are unable to determine, for the factors concerned in the transmission of malaria, at the farm at any rate, were quite favourable. Whatever the reasons may be, they affected Kalerwala and the farm villages equally, in common with other villages in the neighbourhood, and they could not be claimed to have resulted from the reclamation operations. But a clear difference in the salubrity of the control and test villages was observed during 1929-30 which may be ascribed to the relative prosperity at the farm and the treatment available there. As we have already seen, the autumnal malaria epidemic in 1929 and the following spring epidemic were not only much more severe at Kalerwala than at Kot Jan Bakhsh, but the recovery from the disease was undoubtedly slower.

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\*The quotient obtained by dividing the 'fever deaths' in the month of October by the average monthly 'fever mortality' for the preceding April, May, June and July.

## X. CONCLUSIONS.

- (1) The population in the affected villages was subject to chronic malaria, and had low recuperative powers before the reclamation work was started.
- (2) The period of investigation, as well as the universe of description, was relatively small, and observations were made at monthly intervals by a non-resident staff. Final conclusions may not be justified, but it would appear that the reclamation work has been associated with improvement in the health of the inhabitants of the test villages. This population appears to have gained enhanced power of recovery from malaria as compared with the people of the control villages. This has happened without any material change in the factors concerned with the transmission of malaria. It must be assumed that the salubrity is attributable partly to the presence of the dispensary, and partly to the better economic conditions resulting from the reclamation operations.
- (3) The results of this investigation show, if anything, that malaria is more than a mere mosquito problem. They support the original findings of Colonel Gill, *viz.*, that the lowered recuperative power of the Chakanwali villagers on account of poverty and repeated infections was responsible for ill health among these people. They also tend to corroborate the conclusions of the Malaria Commission of the League of Nations (1927), who referring to the Italian system of bonification, or reclamation of marshy lands, say that 'this established value does not lie in the fact that they are essentially anti-larval, indeed, in many instances, they actually increase, by means of their drains, the total area available for anopheline breeding. They are essentially social; they change, that is, a poor, sparse, scattered, often semi-nomadic population into one settled, well-to-do, aggregated into villages with schools, doctors, water supply, proper sewage disposal, and houses of an enforced hygienic standard; and with this change malaria is lessened'.

## XI. RECOMMENDATIONS.

The following additional remedial measures may be recommended :—

- (1) It is desirable that field drainage may be effected by means of porous subsoil pipes instead of by open drains.
- (2) Conditions at the farm are particularly suitable for controlling malaria by regular quininization of infected persons, both tenants and employees, and by administration of plasmochin to those carrying gametocytes.
- (3) The re-establishment of the dispensary at Kot Jan Bakhsh is very desirable.

- (4) Propaganda work to educate the cultivators in personal and communal hygiene may be expected to yield good results.

#### ACKNOWLEDGMENTS.

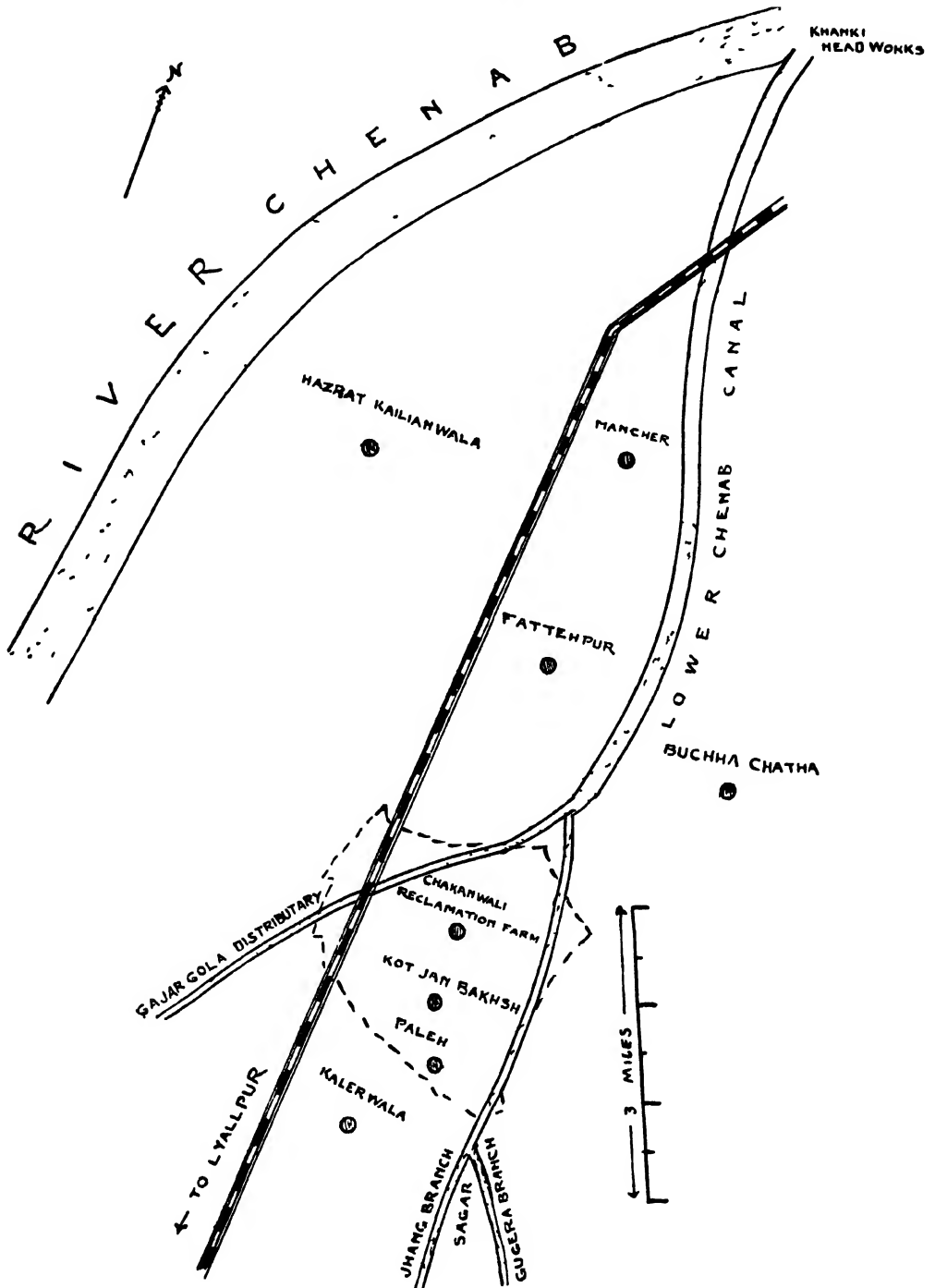
Our gratitude is due to Colonel C. A. Gill, K.H.S., I.M.S., who initiated and guided this investigation. It is our pleasant duty to acknowledge the help and assistance given by our colleagues, Dr. M. Yacob, D.P.H., and Dr. M. L. Bahl, D.P.H., in collecting data at various times. Pandit Daulat Ram, Head Laboratory Assistant, and other laboratory staff rendered valuable assistance both in the field and in the laboratory. We are greatly indebted to Dr. E. McKenzie Taylor, Scientific Research Officer, Irrigation Branch, and to his Assistants, specially Mr. Mukand Lal Mehta, B.Sc., and Sheikh Nazir Ahmed, B.Sc., for kindly placing at our disposal valuable data collected by them.

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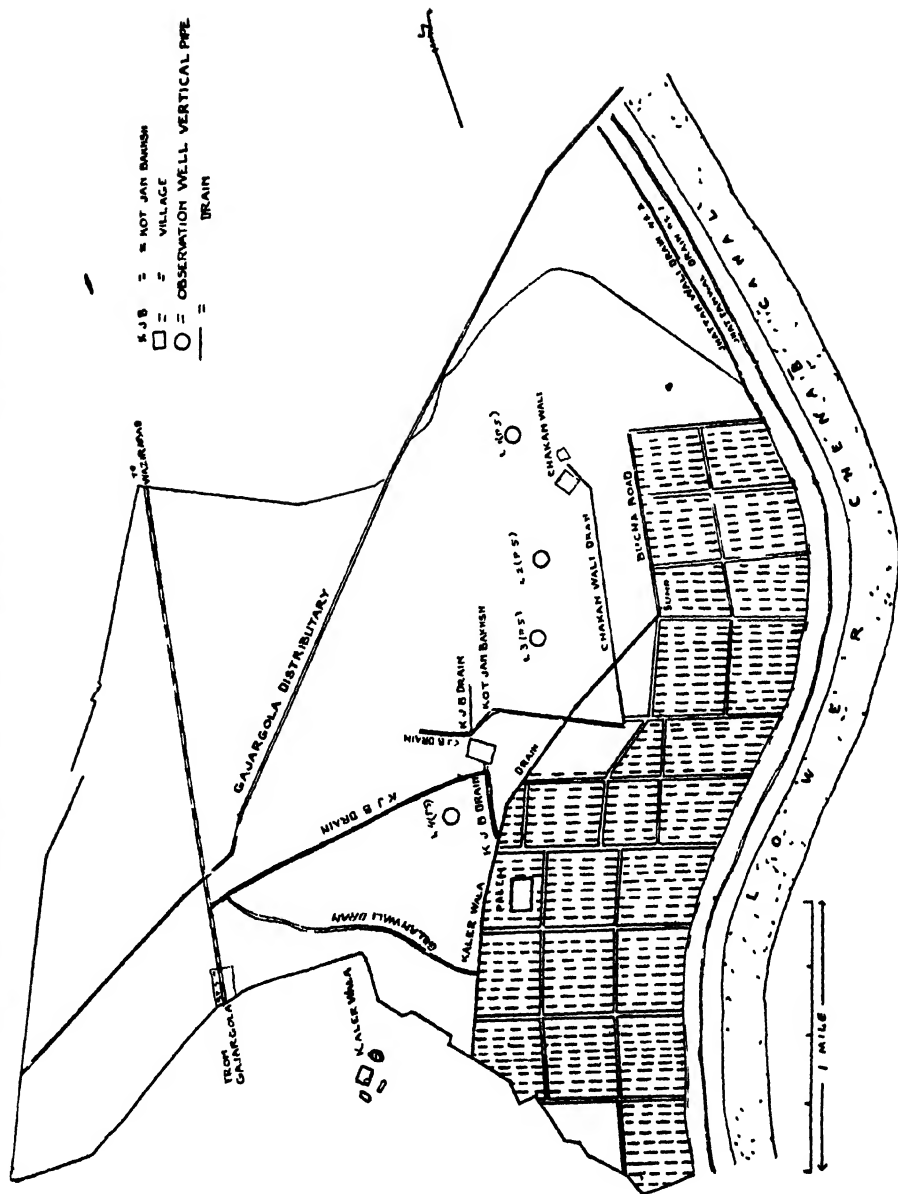
# MAP I.

Showing the Chakanwali Reclamation Farm and its relation to the Lower Chenab Canal





**Showing drainage system and water collections in the farm area and Kalervala.**



## NOTES ON MALARIA IN MYSORE STATE.\*

### Part I.

### THE TOPOGRAPHY, METEOROLOGY AND MALARIAL SEASONS OF MYSORE.

BY

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[June 12, 1933.]

### INTRODUCTION.

THE State of Mysore has a total area of 29,474 square miles lying in the western portion of central South India. It lies between  $11^{\circ} 36'$  and  $15^{\circ} 2'$  north latitude and between  $74^{\circ} 36'$  and  $78^{\circ} 36'$  east longitude. The State is on the southern part of the Deccan plateau, its lowest point being slightly under 2,000 feet above sea level and the highest 6,000 feet above. The greater part of the State, the Maidan, is a gently rolling plain but portions to the west and south, the Malnad, are more hilly.

The rainfall in the State varies greatly, some places having so little as to average less than 16 inches per year, while others along the western border have annual averages of over 200 inches. This rainfall is received during two monsoons, the south-west from April to September, and the north-east from October to March. The average rainfall for the whole State during the south-west monsoon is 27.4 inches, and for the north-east 8.7 inches, a total average rainfall for the State of 36.1 inches per year. Temperature and humidity records are kept in only four cities, Bangalore, Mysore, Hassan and Chitaldrug. The mean maximum temperature in the shade varies in the four places from  $83.5^{\circ}\text{F.}$  at Hassan to  $87.1^{\circ}\text{F.}$  at Chitaldrug, the mean minimum being between  $62.8^{\circ}$  and  $67.5^{\circ}\text{F.}$  at the same two places. Extremes of recorded

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\*The work here reported was done with the support and under the auspices of the Government of Mysore and the International Health Division of the Rockefeller Foundation.

temperature are 42.7° and 103.0°F. The mean humidity per cent varies from 58 at Chitaldrug to 62 at Hassan.

Although malaria had been known to exist in various parts of the State for some years, no extensive study of the situation had been made up to 1927. In that year a rapid spleen survey of children of the State was made and the results incorporated in a report to the Mysore Government. The conclusions of this survey as to the extent of malaria were as follows :—

‘Kolar District is a non-endemic area which has no large endemic foci. Unless it is later found to be subject to epidemic malaria, it may be excluded from areas needing attention’. (It should be noted here that in 1930 one area in this district had a severe epidemic of malaria.)

‘Bangalore and Tumkur Districts have a few endemic foci but are in the main free of serious malaria. Their problems are ones of quite definitely localized areas of infection’.

‘Chitaldrug District contains many endemic areas of light intensity and at least one heavy endemic area’.

‘The remaining four districts have very few non-endemic areas. The endemicity is lightest to the south in the Mysore District and increases towards the north-west to the Kadur and Shimoga Districts’.

At that time nothing was known, except for a few scattering reports, of the anophelines of Mysore, and consequently nothing of the malaria carriers. The chief evidence available as to species of parasites was in reports from three hospitals. Out of 1,985 positive blood examinations recorded in these three hospitals between 1922 and 1927 inclusive, 50.6 per cent were given as benign tertian, 16.7 per cent as malignant tertian, 5.6 per cent as quartan, and 27.1 per cent as mixed infections of species not stated.

Subsequent to the spleen survey of the State, it was decided to select three representative areas for more intensive study and, possibly, later malaria control experiments. The three areas finally selected were: (1) the Nagenhalli area in the Mysore District; (2) the Mudigere area in the Kadur District; (3) the Hiriur area in the Chitaldrug District.

## DESCRIPTION OF STUDY AREAS.

### NAGENHALLI AREA.

The Nagenhalli area centres on the village of Nagenhalli and the Paddy\* Experimental Farm of the Mysore Department of Agriculture. The farm is about a quarter of a mile to the east of the village, which is three miles out of Mysore City and about half a mile to the west of the main Bangalore-Mysore Road. This road passes through the eastern part of the area and the Bangalore-Mysore line of the Mysore Railway passes through the western part, both running north and south. The Nagenhalli Farm has a permanent staff

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\* ‘Paddy’ is the Indian term for rice.

of four including families, subject to the usual Government leave and transfer conditions. The labourers on the farm are recruited from other parts of the Mysore District, probably most frequently from other malarious areas. They vary in number, according to season, between a maximum of 40 and a minimum of 25.

Included in the area are three other villages. Lakshmipur lies one mile to the north of Nagenhalli village along the railway. Kalastvadi and Siddalingapur are on the Bangalore-Mysore Road, one-half mile apart. The former is half a mile from Nagenhalli village, the latter three-quarters of a mile.

The populations of these villages were as follows by the 1931 census :—Nagenhalli Farm and village, 900; Lakshmipur, 323; Kalastvadi, 132; Siddalingapur, 229. This population, being either owners, tenants or labourers of local land, is very stable and travels little.

The whole area is gently rolling country, with a low range of hills to the west and a general gradual slope towards the east. It is at an average of about 2,450 feet above sea level, and is in the part of the State which receives an average of from 25 to 40 inches of rain per annum.

Practically the whole of the area is under 'paddy' cultivation, watered by means of irrigation channels derived from the Cauvery River. The area is, however, too far from the river for it to be a factor in producing malaria-carrying mosquitoes. There are two main irrigation channels in the area, one to the south and the other flowing through the northern part. Two larger subsidiaries flow through the centre, one forming the southern boundary of Nagenhalli village and flowing on eastward to pass through the farm, the other flowing approximately north and south through the farm. There are, of course, very numerous smaller channels running to separate areas of paddy cultivation and between the smaller plots.

Paddy is grown between the first of June and the end of the year, so that during these months channels of all sizes run full continuously and the fields are under slowly flowing water. Between the first of January and the first of June, the fields lie fallow and the channels dry up, with the exception that for the first ten days of each month water is turned into the main and first subsidiary channels. As a result, these channels are seldom completely dry. All the channels are much silted up and full of vegetation during the irrigation season, attempts to clean them out being irregular and poorly supervised. No efficient scheme of drainage is in existence, and the railway and main road obstruct such natural drainage as exists, so that there are many marshy areas not cultivated. In addition to seepage from the channels, the water has been allowed in places to back up into nullahs across the course of which the channel cuts, and ponds with marshy edges are thus formed.

The whole area investigated was originally taken on the basis of a circle of one mile radius with Nagenhalli village and farm as a centre. The malaria survey began in this area on 1st October, 1928.

**MUDIGERE AREA.**

The Mudigere area is in the Kadur District, 19 miles to the south-east of Chikmagalur and 44 miles from Kadur,\* along the main Kadur-Mangalore Road which runs through the town of Mudigere, a minor municipality. The town is on the southern end of a ridge, which extends from a range of hills to the north and runs two miles south between two valleys, which meet at the end of the ridge. At the upper end of the town, the ridge is 120 feet above the floor of the eastern valley and 75 feet above the western valley. Both of these valleys contain streams, the one to the east being broader and more quietly flowing, while the one to the west runs on a rocky floor, very swiftly in most parts, with two small waterfalls. This latter stream turns to the west away from the town early in its course. Water from both streams is used to irrigate the paddy fields filling the floor of the valleys, which vary from one to one-quarter mile in breadth and have steep hills on the sides away from town. There are many seepages along both sides of the town ridge, the water from which is used to assist in the irrigation of the paddy fields. On the other side of the eastern valley there are five small intersecting valleys facing the town which, besides paddy fields, contain extensive marshes.

To the south-east of the town ridge, after the western valley stream has turned away, there is a large, much overgrown tank three-quarters of a mile from Mudigere. This furnishes water for two channels which irrigate paddy fields to the west and south of the town. These fields cover the valley at the end of the ridge and merge with those irrigated by the eastern stream.

The town ridge slopes gradually to the south, and merges into a narrow valley at its southern point where the houses are only 30 to 50 feet above the paddy fields. Mudigere averages 3,195 feet above sea level, and lies in that part of the State which has an average of from 60 to 100 inches of rain per annum.

The population of Mudigere by the 1931 census was 1,648. Although this population is rather stable, the growth of a bus service on the main road has increased the travel habits of the people. Across the valley to the west and south are two coffee estates employing labourers imported from the west coast, who, however, are present on the estates for part of the year only, and mix but little with the town population.

Included in the area are two villages. The nearest to the town, and situated on the hills across the narrow valley to the south of it, is Old Mudigere. Its population in 1931 was 359. A portion of this village is practically in one of the coffee estates, and the contact of the villagers with imported labourers is more intimate than is the case in the town. Hesgal, the other village, lies three-quarters of a mile to the north-west of Mudigere town and across the valley on the opposite slope. The villagers here are more isolated, and have

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\* The last named town is on the Bangalore-Hubli-Poona branch of the Madras and Southern Mahratta Railway.

very little contact with even the people of the town, except on days of the weekly market. The population of Hesgal in 1931 was 259.

In this area one crop of paddy is grown between June and December of each year. For the remaining six months the fields are dry. Both of the streams in the two main valleys are perennial, and do not dry up, but the two channels from the tank to the south-east of the town are dry in the season when paddy is not grown. The malaria survey in this area began in January 1929.

#### HIRIYUR AREA.

The Hiriyr area is in the south-eastern part of the Chitaldrug District on the main Bangalore-Bellary Road, 99 miles from Bangalore. The town of Hiriyr, with a population of 3,536 by the 1931 census, is a minor municipality and a taluq headquarters. It lies on the right bank of the Vedavati River, which at this point is 1,965 feet above sea level. The average rainfall of this part of the State is below 25 inches.

In 1898 a large dam was built on the Vedavati River 12 miles above the town of Hiriyr. It is not possible to get figures which might indicate the status of malaria in the town previous to the building of the dam, but the tradition is that the town was healthy. It seems probable, from conditions elsewhere, that previous to the building of the dam the river had a sandy bed and was completely dry for a considerable part of the year. At present it is heavily silted and covered with a heavy growth of aquatic and marsh vegetation, through which a narrow channel carries a main stream that is never dry. Following rains and when the water is in the irrigation channels, the river is moderately flooded; between such occurrences practically the whole bed is a marsh. The bed of the river is from 10 to 20 feet below the level of the surrounding plain.

On either side of the river, at distances varying from one-quarter to two miles, are two large irrigation channels. Water from the channel on the north bank is used to irrigate sugar-cane, plantains and, to a small extent, paddy fields. The south bank channel irrigates paddy fields to the west of the town, and also some acres of sugar-cane and plantains. No attention has been paid to draining this water into the river, and it finds its way through numerous small and larger nullahs which empty into the river, creating many marshes along such nullahs. Water flows in the irrigation channels at various intervals depending on the amount of water available at the dam. During the first year's work in this area, water was turned into the channels for seven and off for ten days. Since 1st January, 1929, when work was begun in this locality, the area under paddy cultivation has materially increased.

There are two villages in the area in addition to Hiriyr town. Huchav-vanhalli, with 280 population in 1931, is one mile from Hiriyr town to the north of the river and distant one and one-half miles from it. Babbur is also

to the north of the river, one mile distant, and is on a subsidiary of the left-bank main channel. In 1931 it had a population of 1,314. The people of all three places constitute a very stable population amongst whom immigration or emigration is rare. Practically all of the surrounding areas, to which these people might make visits, are either less malarious or free of malaria.

### STAFF AND WORK OF THE STATIONS.

The staff of each of these three stations consisted at first of one third-class health officer in charge, three assistant sanitary inspectors, one clerk and three peons. All of these men were locally recruited and trained in their duties.

Each station was considered to consist of the named village or town as the centre of a circle with a one-mile radius. In some instances the circle

TABLE I.  
*Rainfall records of Nagenhalli station (inches).*

Months.	* Average rainfall 33 years.	1929.			1930.			1931.		
		Rainfall.	Maximum in 24 hours.	Number of days of rain.	Rainfall.	Maximum in 24 hours.	Number of days of rain.	Rainfall.	Maximum in 24 hours.	Number of days of rain.
January ..	0.15	0.71	0.59	2	0.45	0.45	1	0.05	0.05	1
February ..	0.16	0.13	0.08	2	0.00	0.00	0	0.00	0.00	0
March ..	0.48	0.21	0.21	1	0.66	0.66	1	0.23	0.23	1
April ..	2.44	4.00	1.21	11	0.35	0.35	2	1.20	0.49	6
May ..	5.09	3.51	1.06	11	12.41	1.47	9	2.44	1.02	5
June ..	2.71	2.37	0.57	13	0.37	0.30	2	1.46	0.57	5
July ..	2.69	1.32	0.30	7	0.96	0.65	3	1.30	0.79	7
August ..	3.11	1.29	0.43	7	0.28	0.25	2	1.36	0.93	9
September ..	4.72	6.96	2.90	11	3.13	1.55	10	6.04	1.42	10
October ..	5.93	2.21	0.63	7	9.27	1.18	17	1.67	1.00	2
November ..	2.58	0.77	0.33	5	3.36	1.43	6	7.58	2.17	9
December ..	0.34	0.27	0.25	2	0.51	0.41	2	1.19	1.10	2
For year ..	30.43	23.78	2.90	79	31.75	1.55	55	24.52	2.17	57

\* Average is for Mysore City, four miles from Nagenhalli.

TABLE II.

*Rainfall records of Mudigere station (inches).*

Months.	Average rainfall 35 years.	1929.			1930.			1931.		
		Rainfall.	Maximum in 24 hours.	Number of days of rain.	Rainfall.	Maximum in 24 hours.	Number of days of rain.	Rainfall.	Maximum in 24 hours.	Number of days of rain.
January ..	0.14	0.15	0.15	1	0.31	0.31	1	0.00	0.00	0
February ..	0.10	0.00	0.00	0	0.00	0.00	0	0.00	0.00	0
March ..	0.45	0.00	0.00	0	0.54	0.54	1	0.26	0.26	1
April ..	2.44	7.22	1.40	14	1.58	0.86	2	5.69	2.33	8
May ..	4.68	4.83	1.40	11	5.33	1.61	9	1.85	0.81	9
June ..	19.16	21.59	3.70	27	21.01	5.90	19	9.70	1.54	24
July ..	30.77	30.96	8.30	30	14.32	4.37	26	35.00	5.03	31
August ..	16.58	9.49	1.00	26	8.76	1.55	24	41.57	5.85	31
September ..	9.14	8.77	1.20	19	9.45	2.03	20	7.26	1.74	18
October ..	8.40	5.74	1.00	12	10.05	2.13	16	1.90	0.40	10
November ..	3.11	3.30	1.80	5	0.79	0.38	4	3.80	1.53	10
December ..	0.68	0.00	0.00	0	0.04	0.04	1	3.23	1.73	5
For year ..	95.05	92.05	8.30	145	72.18	5.90	123	110.26	5.93	147

included the adjacent villages mentioned, but in others such villages were beyond the radius. In the centre and in the adjacent villages, certain houses and cattle-sheds were selected as permanent catching stations of adult anophelines.

The work of the staff was assigned on a definite routine which called for work in the laboratory every afternoon except Saturday. On Monday mornings one of the centres was visited for taking blood smears and making spleen examinations. The work was so arranged that each central town and each of the outlying villages was visited once a month for this purpose. On Tuesday mornings a survey of the area was made for anopheline larvæ. On Wednesday and Friday mornings the fixed catching stations were visited, and adult anophelines captured. The mornings of Thursday and Saturday were given



over to laboratory work, consisting of examination of blood slides, identification of larvæ and their breeding out, identification and preservation of adult anophelines, and dissection of suitable specimens of female anophelines.

### RAINFALL AND TEMPERATURE RECORDS.

Rainfall records were available for each of the three stations selected. In the case of Nagenhalli station the records were maintained by the Experimental Farm of the Mysore Department of Agriculture. Records at the other two stations had been maintained by the municipal authorities. The Nagenhalli station is approximately four miles from the observatory in Mysore City, where records are available for the past 35 years. The average rainfall of each of the three stations, and records for 1929 to 1931 are given in Tables I, II and III.

TABLE III.

*Rainfall records of Hiriya station (inches).*

Months.	Average rainfall 35 years.	1929.			1930.			1931.		
		Rainfall.	Maximum in 24 hours.	Number of days of rain.	Rainfall.	Maximum in 24 hours.	Number of days of rain.	Rainfall.	Maximum in 24 hours.	Number of days of rain.
January ..	0.08	0.00	0.00	0	0.35	0.35	1	0.00	0.00	0
February ..	0.10	0.00	0.00	0	2.25	2.25	1	0.00	0.00	0
March ..	0.20	0.00	0.00	0	0.00	0.00	0	0.00	0.00	0
April ..	0.97	1.40	0.43	4	1.34	1.54	1	0.54	0.44	2
May ..	3.01	5.40	1.90	5	2.82	1.77	5	0.11	0.09	2
June ..	2.04	0.53	0.53	1	1.00	0.75	3	1.49	0.92	3
July ..	1.86	0.85	0.85	1	0.39	0.12	4	0.00	0.00	0
August ..	1.56	0.63	0.20	4	1.25	0.70	5	0.43	0.43	1
September ..	4.05	10.82	3.76	9	6.44	2.80	7	4.44	1.02	8
October ..	3.59	3.49	1.12	7	10.08	4.21	7	0.69	0.47	3
November ..	1.80	2.39	1.29	4	0.60	0.60	1	4.81	2.43	7
December ..	0.28	0.00	0.00	0	0.34	0.34	1	1.75	1.38	4
For year ..	19.54	25.51	3.76	35	27.06	4.21	36	14.26	2.43	30

The Government of Mysore maintains four observatories at which temperature records are available, but none of these, except possibly the one at Mysore City, was close enough to any of the stations to make their records of any great value in our work. Maximum, minimum, wet and dry bulb thermometers were put in Nagenhalli with the beginning of work there; dry and wet bulb thermometers were placed at Mudigere on 18th February, 1929, and maximum and minimum thermometers on 11th May; all four types were installed at Hiriur on 24th April, 1929. It was unfortunately not possible to house these thermometers in the proper way, so they were placed in the best available situations on roofed verandahs, in entrance ways, or in rooms. Temperatures recorded by them represent household conditions rather than atmospheric. Certain of the temperature readings and the average 8 a.m. relative humidities are given in Tables IV, V and VI.

TABLE IV.

*The maximum and minimum temperatures registered, the mean temperature and the average 8 a.m. relative humidity at Nagenhalli station.*

Months.	1929.				1930.				1931.			
	Maximum temperature registered (°F.).	Minimum temperature registered (°F.).	Mean temperature (°F.).	Average 8 a.m. humidity. (per cent).	Maximum temperature registered (°F.).	Minimum temperature registered (°F.).	Mean temperature (°F.).	Average 8 a.m. humidity (per cent).	Maximum temperature registered (°F.).	Minimum temperature registered (°F.).	Mean temperature (°F.).	Average 8 a.m. humidity (per cent).
January	89.0	58.0	75.3	80.2	92.0	58.0	75.9	70.1	91.0	60.0	75.3	84.0
February	93.0	67.0	79.7	76.6	98.0	62.0	78.4	72.1	94.0	62.0	79.0	81.2
March ..	98.0	67.0	82.6	66.2	98.0	63.0	82.7	70.0	98.0	67.0	83.5	66.9
April ..	98.0	69.0	81.4	71.6	100.0	70.0	84.6	61.4	100.0	72.0	83.9	63.0
May ..	94.0	69.5	79.1	71.2	97.0	69.0	80.1	69.4	100.0	68.0	81.8	69.0
June ..	82.0	62.0	73.1	76.9	91.0	67.0	75.1	70.4	89.0	69.0	75.5	74.5
July ..	89.5	62.0	72.6	77.5	85.0	66.0	73.8	74.0	82.0	67.0	72.1	82.5
August ..	85.0	62.0	73.4	73.7	90.0	66.0	74.9	75.3	80.0	68.0	71.4	79.6
September	92.0	65.0	75.7	75.5	94.0	63.0	75.5	78.7	89.0	64.0	74.3	78.6
October	91.0	65.0	75.2	82.5	89.0	66.0	76.9	84.7	90.0	65.0	76.9	77.6
November	88.0	65.0	75.8	84.9	90.0	62.0	76.0	80.7	94.0	62.0	76.1	81.1
December	89.0	59.0	75.0	79.6	89.0	58.0	74.7	81.1	85.0	59.0	72.9	79.3
For year	98.0	58.0	76.6	76.3	100.0	58.0	77.4	74.0	100.0	59.0	76.9	76.4

TABLE V.

*Temperature and relative humidity records of Mudigere station.*

Month.	1929.				1930.				1931.			
	Maximum temperature registered (°F.).	Minimum temperature registered (°F.).	Mean temperature (°F.).	Average 8 a.m. humidity (per cent).	Maximum temperature registered (°F.).	Minimum temperature registered (°F.).	Mean temperature (°F.).	Average 8 a.m. humidity (per cent).	Maximum temperature registered (°F.).	Minimum temperature registered (°F.).	Mean temperature (°F.).	Average 8 a.m. humidity (per cent).
January ..	..	..	..	..	98.0	55.0	76.2	76.6	95.0	58.5	76.8	77.1
February ..	..	..	..	73.2	98.0	55.0	76.1	71.3	97.5	62.0	79.9	79.0
March ..	..	..	..	73.6	98.0	63.0	80.5	78.9	97.5	63.5	80.0	76.6
April ..	..	..	..	85.2	98.0	65.0	80.9	84.4	96.0	66.0	78.0	86.1
May ..	89.5	68.0	77.1	88.2	90.0	65.0	75.7	90.7	94.0	65.0	78.7	86.6
June ..	80.0	61.0	70.0	93.5	89.0	63.0	72.1	91.5	81.5	64.0	71.9	92.5
July ..	75.0	64.0	69.1	94.8	77.0	64.0	69.4	94.0	77.0	64.0	69.4	96.0
August ..	77.0	64.0	69.4	94.8	77.0	64.0	69.7	96.0	76.0	65.0	69.2	96.5
September	84.0	63.0	72.6	92.8	86.0	62.0	71.8	92.4	84.0	64.0	71.2	93.3
October ..	88.0	64.0	73.8	89.9	88.0	65.0	74.7	89.3	90.5	63.0	74.0	89.1
November ..	92.0	69.0	74.6	83.5	91.5	60.0	75.2	79.7	90.0	60.5	75.9	84.6
December	92.0	59.0	75.3	80.4	93.0	57.0	76.0	81.6	90.5	55.0	73.2	83.1
For year ..	..	..	..	..	98.0	55.0	74.9	85.5	97.5	55.0	74.8	86.7

The three stations chosen were in three quite different rainfall zones, the average annual rainfall being 30, 96 and 19 inches respectively. Of the three years given in the tables, 1929 and 1931 were deficient in rainfall in Nagenhalli, while 1930 was above the average at that station. In both Mudigere and Hiriur, 1929 and 1930 were below average and 1931 was above. The differences in rainfall in the three stations are further shown by the number of days of rain per year, the average figure for Hiriur being 34 per year, as against 64 and 138 for Nagenhalli and Mudigere respectively.

The differences in temperature of the three stations were not so great as might possibly have been expected. For the years under review, the maximum temperature varied from 98°F. in Mudigere to 102°F. in Hiriur, and the minimum temperature from 55.0° to 61.0°F. in the same two stations. The

TABLE VI.

*Temperature and relative humidity records of Hiriyr station.*

Months.	1929				1930				1931			
	Maximum temperature registered (°F.).	Minimum temperature registered (°F.).	Mean temperature (°F.).	Average 8 a.m. humidity (per cent).	Maximum temperature registered (°F.).	Minimum temperature registered (°F.).	Mean temperature (°F.).	Average 8 a.m. humidity (per cent).	Maximum temperature registered (°F.).	Minimum temperature registered (°F.).	Mean temperature (°F.).	Average 8 a.m. humidity (per cent).
January ..	..	..	..	..	86.0	64.0	75.7	73.3	85.0	64.0	75.4	69.4
February ..	..	..	..	..	90.0	64.0	78.2	66.9	91.0	67.0	80.1	60.1
March ..	..	..	..	..	95.0	67.0	84.2	63.5	96.0	75.0	86.0	54.3
April ..	..	..	..	..	98.0	76.0	84.9	72.9	99.0	79.0	80.1	69.1
May ..	96.0	75.0	85.4	74.7	96.0	73.0	84.9	77.6	102.0	77.0	88.6	65.7
June ..	91.0	72.0	81.4	76.8	94.0	73.0	83.4	77.1	94.0	73.0	83.4	75.5
July ..	87.0	73.0	79.7	78.2	89.0	73.0	90.3	79.8	90.0	73.0	80.9	76.5
August ..	88.0	73.0	80.2	77.9	88.0	73.0	79.9	75.8	88.0	73.0	80.2	78.8
September	89.0	73.0	80.6	79.5	89.0	73.0	80.5	79.3	89.0	72.0	79.7	80.2
October ..	87.0	73.0	79.5	79.3	87.0	73.0	78.7	84.9	91.0	70.0	81.5	71.8
November	86.0	69.0	77.1	81.6	87.0	65.0	76.0	80.3	89.0	69.0	77.9	81.4
December	83.0	65.0	75.7	78.4	86.0	64.0	75.3	76.1	84.0	61.0	73.9	84.2
For year ..	..	..	..	..	98.0	64.0	80.2	75.6	102.0	61.0	81.4	72.2

yearly average mean temperature was 77.0°F. in Nagenhalli, 74.8°F. in Mudigere and 80.8°F. in Hiriyr. The greatest differences occurred in the 8 a.m. relative humidities, the averages being 75.6 per cent for Nagenhalli, 86.1 for Mudigere and 73.9 for Hiriyr.

It was difficult to determine any definite seasons for the three stations. The following divisions of the year were made for the purposes of this study.

- I. Hot and dry—February, March, April and May.
- II. Cooler and with highest rainfall—June, July, August and September.
- III. Cool and with second highest rainfall—October, November, December and January.

Table VII gives the average mean temperatures, average 8 a.m. relative humidities, with average and actual rainfalls for these three groups of months.

In the Mudigere and Hiriyur figures, where temperatures and humidities were lacking for certain months in 1929, the average of the 1930 and 1931 figures has been used.

TABLE VII.

*Average mean temperatures, average 8 a.m. relative humidities with average and actual rainfalls for three groups of months.*

Stations.	Groups of months.	Average mean temperatures (°F.)	Average 8 a.m. humidities (per cent).	RAINFALL. (Inches).	
				Average for 35 years.	Actual.
Nagenhalli, 1929	I February, March, April and May.	80.4	70.2	8.17	7.88
	II June, July, August and September.	73.7	75.9	13.26	11.94
	III October, November, December and January.	75.5	79.3	9.00	3.70
Mudigere, 1929 ..	I	78.2	79.3	7.67	12.05
	II	70.3	94.0	75.65	70.81
	III	75.0	82.6	12.33	9.35
Hiriyur, 1929 ..	I	83.9	67.9	4.28	6.80
	II	80.5	78.1	9.51	12.83
	III	77.0	78.1	5.75	6.23
Hiriyur, 1930 ..	I	83.0	70.2	..	6.61
	II	81.0	78.0	..	9.08
	III	76.3	77.7	..	11.02

### DEATH AND DISPENSARY RECORDS.

Deaths in Mysore State are reported through the revenue staff and are somewhat less than fifty per cent accurate as regards number. They are reported under eight general causes, the reported cause being almost always determined by laymen. Of the eight reported causes, it seems probable that 'fevers' represents the best classification to use in an effort to approximate the deaths due to malaria. In spite of the admitted inaccuracy of these reports,

certain figures are given here for what they may be worth. It is of interest to know that, for the State as a whole, about forty-eight per cent of the reported deaths are given as due to fever.

There are dispensaries in the towns of Mudigere and Hiriyr, but none in Nagenhalli village. Figures were available from these two dispensaries for the diagnoses of malaria made among the total number of patients attending the dispensary. These diagnoses, probably without exception, were made clinically—without laboratory confirmation—and are subject to the usual errors of such diagnoses. Table VIII gives the percentage of fever deaths to total deaths reported, and the percentage of malaria diagnoses to the total number of patients attending the dispensaries, for the past five years.

TABLE VIII.

*Percentage of fever deaths to total deaths reported, and percentage of malaria diagnoses to total patients attending dispensaries for the years 1925 to 1930.*

Years.	MUDIGERE.		HIRIYUR.		NAGENHALLI.
	Percentage of fever deaths to total deaths reported.	Percentage of malaria diagnoses in total patients of dispensary.	Percentage of fever deaths.	Percentage of malaria diagnoses.	Percentage of fever deaths.
1925 ..	76.7±5.2	42.2±0.4	..	37.2±0.3	69.6±6.5
1926 ..	68.1±3.7	60.2±0.3	66.6±4.1	32.4±0.3	21.2±4.8
1927 ..	75.4±3.8	55.2±0.3	47.9±4.9	38.3±0.2	37.5±8.2
1928 ..	92.6±2.0	50.8±0.4	73.5±3.6	33.8±0.3	90.9±5.8
1929 ..	83.3±4.2	37.9±0.4	47.6±4.2	32.2±0.3	31.8±4.7
1930 ..	..	..	60.5±5.3	30.7±0.2	..
All years ..	79.7±1.6	50.2±0.2	59.9±2.0	34.1±0.1	41.7±2.9

It may be of interest to note that the highest percentages of deaths in all three places were in 1928, and that in 1926 this percentage was the lowest in two stations. The percentage of malaria diagnoses was highest during 1926 in Mudigere and during 1927 in Hiriyr, and was lowest during 1929 in Mudigere and during 1930 in Hiriyr. It is probably wise, however, not to press these apparent similarities too far.

The reported deaths for the five years in each of the three stations were divided by months in which the report was made. The resultant percentages

were so nearly alike, when the probable errors were considered, that no information could be gained from this classification. The same classification was made for the reported malaria diagnoses in the dispensaries of Mudigere and Hiriya. Table IX gives the results of this classification for the three groups of months previously mentioned. The figures for Mudigere are a total for the five years 1925 to 1929 inclusive, and those for Hiriya for six years 1925 to 1930 inclusive.

TABLE IX.

*Percentage of patients attending Mudigere and Hiriya dispensaries diagnosed as having malaria, by three groups of months.*

		I February, March, April and May.	II June, July, August and September.	III October, November, December and January.	TOTALS.
Mudigere dispensary, 1925-1929 inclusive.	Total patients ..	14,412	13,311	14,625	42,348
	Number diagnosed as malaria.	6,821	7,098	7,331	21,250
	Per cent malaria	47.3±0.3	53.3±0.3	50.1±0.3	50.2±0.2
Hiriya dispensary, 1925-1930 inclusive.	Total patients ..	26,115	28,274	29,075	83,462
	Number diagnosed as malaria.	9,338	7,564	11,523	28,425
	Per cent malaria	35.8±0.2	26.8±0.2	39.6±0.2	34.1±0.1

The diagnosis rates for the three groups of months in both the Mudigere and Hiriya dispensaries were significantly different from each other. In Mudigere the highest rate was in group II and the lowest in group I, whereas in Hiriya the highest rate was in group III and the lowest in group II.

### SPLEEN RATES.

Spleen and parasite rates were determined once a month in each town and village in or near each area, with the exception of the Hiriya area. In the latter area spleen and blood examinations were made once a month in Hiriya town, but only once a quarter in Babbur and Huchavvanhalli villages, so the latter figures were not used. From the Nagenhalli area figures, examinations of the labourers of the Nagenhalli Experimental Farm have been excluded as they were a transitory population. When this work began, it was early found that it was not possible to control this civilian population so as to allow of any

definite scheme of examination. For this reason it was decided to treat each month's examinations as a new sample of the population with no necessary reference to previous examinations. On this basis an effort was made each month to make an examination of a fair sample of the town or village population. The rates determined from these examinations have the errors inherent in such a system, when compared with one in which some definite regimentation of the population is possible.

For the present the spleen and parasite rates of the 0-9 year age-group only will be used, and a more detailed report of spleen and blood findings will be made later. The 0-9 year age-group was really one from two or three years to nine years, as it was rarely possible to get permission for the examination of children under two years. However, since such examinations were so few as not appreciably to affect the general results, they were not removed from the figures considered. The rates given include figures for both sexes.

The spleen rates given here are the percentages of children showing enlarged spleens on examination, regardless of the size of the spleen. All spleen examinations were made with the patient lying down and knees drawn up. Table X

TABLE X.

*Spleen rates of children 0-9 years old by months and years in three stations.*

Months.	NAGENHALLI.		MUDIGERE.		HIRIYUR.	
	1928.	1929.	January 1930 and Decem- ber 1928.	1929.	1929.	1930.
January ..	..	94.5±2.1	65.1±4.9	83.7±3.6	45.8±6.9	66.7±8.2
February ..	..	90.0±2.9	..	69.2±2.6	56.3±5.3	75.6±4.3
March ..	..	87.1±4.1	..	85.6±2.7	57.1±6.3	72.0±6.1
April ..	..	85.3±4.1	..	82.0±3.3	62.5±5.2	75.0±5.2
May ..	..	76.9±4.6	..	85.6±2.4	70.6±7.5	61.1±5.4
June ..	..	81.6±4.2	..	79.1±4.2	44.0±6.7	38.9±7.7
July ..	..	73.7±6.8	..	91.2±3.3	..	36.4±9.8
August ..	..	64.3±5.0	..	90.7±3.0	50.0±23.8	..
September ..	..	77.8±5.5	..	85.7±4.0	30.0±4.4	38.1±7.1
October ..	89.2±2.3	82.6±5.3	..	62.9±5.5	35.4±4.7	..
November ..	90.7±3.0	79.3±5.1	..	70.7±3.4	45.8±6.9	36.9±4.8
December ..	85.7±3.0	84.2±5.6	93.9±2.0	72.4±4.0	57.1±8.9	41.7±9.6
All months ..	83.9±1.0		78.6±0.9		47.9±1.9	57.3±2.1



gives the spleen rates of the 0-9 year age-group by months and years for all examinations made in the Nagenhalli and Mudigere areas and in Hiriya town.

Considering the spleen rates for all months, we find that the four rates are all significantly different from each other. The differences are as follows :—

Nagenhalli and Mudigere areas =  $5.2 \pm 1.3$ .

Nagenhalli and Hiriya, 1929 =  $36.0 \pm 2.1$ .

Nagenhalli and Hiriya, 1930 =  $26.6 \pm 2.3$ .

Mudigere and Hiriya, 1929 =  $30.7 \pm 2.1$ .

Mudigere and Hiriya, 1930 =  $21.3 \pm 2.3$ .

Hiriya, 1929, and Hiriya, 1930 =  $9.4 \pm 2.8$ .

As a further demonstration of the differences in the spleen rates of the three areas the enlarged spleens were divided into two groups, (a) those palpated at the border of the ribs, or within one-third of the distance between the border of the ribs and the umbilicus, and (b) those palpated below one-third of this distance. In the Nagenhalli area what may be called the small-spleen rate was  $33.3 \pm 1.3$  and the large-spleen rate was  $50.6 \pm 1.4$ . The same rates for the Mudigere area were, respectively,  $41.9 \pm 1.1$  and  $36.7 \pm 1.1$ ; for Hiriya, 1929, they were  $32.8 \pm 1.8$  and  $15.1 \pm 1.4$ ; for Hiriya, 1930,  $43.9 \pm 2.0$  and  $13.4 \pm 1.4$ . The large-spleen rates of the three areas were significantly different from each other in the same order as were the general spleen rates. The large-spleen rates of Hiriya town for 1929 and 1930 were not significantly different from each other. The highest small-spleen rates were the  $41.9 \pm 1.1$  of Mudigere and  $43.9 \pm 2.0$  of Hiriya, 1930, which did not differ significantly. The small-spleen rates of Nagenhalli and Mudigere were significantly different, the Nagenhalli rate being the lower. The small-spleen rates of Nagenhalli and Hiriya, 1929, were the same, but the Hiriya rate in 1930 was significantly higher. The entire significant difference between the 1929 and 1930 general spleen rates in Hiriya was accounted for by the significant increase of the small-spleen rate for 1930 over that for 1929.

A consideration of the monthly spleen rates of the Nagenhalli area shows that the rates for October, November and December 1928 were not significantly different from those of the three corresponding months of 1929. The rates were all fairly close and show comparatively little variation. The rate for August, however, was significantly lower than that for January.

In the Mudigere figures the rate for December 1928 is higher than that for December 1929, and the rate for January 1930 lower than that for January 1929. It would appear that there was some condition operating to increase spleen rates towards the end of 1928, which did not exist in the last months of 1929. This condition had ceased to operate by February 1929, as the rate for this month is significantly lower than that for January 1929. It seems probable that the January 1930 rate is more nearly representative of usual conditions. The rate for March is significantly higher than that for February,

and possibly represents the beginning of the 'season'. The rate for October is significantly below that for September.

Due to the smaller number of examinations in Hiriyr town, and the consequent large probable errors, the monthly rates for 1929 were not significantly different from those for 1930. The rate given for August 1929 was not significant, so there were no available rates for the month of August.

As mentioned above the spleens found enlarged were classified as to size, so that a further consideration of monthly incidence may help to define a malaria season, as far as spleen rates can be used for this purpose. To obtain figures of more significance, the months are combined into the three seasons mentioned in the discussion of rainfall and temperature. Tables XI and XIa give a small-spleen rate and a total spleen rate found at examinations made during the three groups of months. In Nagenhalli and Mudigere the figures are for examinations of children between 0 and 9 years of age, and are for the months of 1929 only. In order to obtain more significant results, the results of all examinations made in Hiriyr in 1929 and 1930 are used in Tables XI and XIa, not those of children only.

TABLE XI.

*Small-spleen rates for different seasons in three stations.*

Groups of months.			NAGENHALLI.	MUDIGERE.	HIRIYUR.	
			0-9 years.	0-9 years.	All ages.	
			1929.	1929.	1929.	1930.
			Small-spleen rate.	Small-spleen rate.	Small-spleen rate.	Small-spleen rate.
F. M. A. M.	I	..	31'8±2'5	43'7±1'7	42'9±2'0	50'0±2'2
J. J. A. S.	II	..	30'9±2'8	41'9±2'7	26'9±1'9	29'9±2'4
O. N. D. J.	III	..	26'4±2'6	41'5±2'2	33'8±2'1	22'3±1'9
For year	..	..	29'9±1'5	42'7±1'2	34'8±1'2	34'6±1'3

The small-spleen rates for the Nagenhalli area are all the same, the differences not being sufficiently larger than their probable errors. The total spleen rates for seasons I and III are the same, but are both significantly higher than the same rate for season II. From these figures it seems possible that the malaria season extends from October to May, with some indication that the latter months of the year are the most dangerous.

TABLE XIa.

*Total spleen rates for different seasons in three stations.*

Groups of months.	NAGENHALLI.	MUDIGERE.	HIRIYUR.	
	0-9 years.	0-9 years.	All ages.	
	1929.	1929.	1929.	1930.
	Total spleen rate.	Total spleen rate.	Total spleen rate.	Total spleen rate.
F. M. A. M. I ..	85.1±1.9	78.7±1.4	56.7±2.0	68.0±2.1
J. J. A. S. II ..	73.8±2.6	86.4±1.8	38.5±2.1	39.6±2.6
O. N. D. J. III ..	87.2±2.0	72.8±2.0	51.7±2.2	36.4±2.2
For year .. ..	82.2±1.2	78.5±1.0	49.2±1.2	49.0±1.4

The same situation is true of the small-spleen rates of the Mudigere area, but in this area the total spleen rate for season II is significantly higher than those for seasons I and III which are the same. It seems possible that the malaria season in this area may start in the early months of the year and culminate between June and September.

In Hiriya, where two years figures were available before control work started, it was found that the small-spleen rates for corresponding seasons of 1929 and 1930 were the same, except for season III where this rate was significantly lower in 1930. The total spleen rate for season I was higher in 1930 than in 1929, and significantly lower in 1930 for season III. In 1929 the small-spleen rate for season I was significantly higher than the rates for seasons II and III, the latter two rates not being different. The same finding was true for 1930. The total spleen rate of season I was not different from that of season III in 1929, but both were higher than that for season II. In 1930, however, the total spleen-rate for season I was higher than the rates for the other two seasons, the rates for which were not different. It seems possible from these findings that the malaria season in Hiriya is from February to May, and that the last months of the year may be included or not, according to variations in the factors governing the transmission and recurrence of malaria.

### PARASITE RATES.

From each person examined for enlarged spleen, a blood smear was taken, if possible, therefore the remarks above as to selection of sample apply to parasite rates as well as to spleen rates. Both thick and thin smears were

made from each person. In the earlier stages of the work the examiners depended very largely on the thin smears for their diagnoses, but as experience increased the results depended more and more on examination of thick smears. The slides were stained with Giemsa stain and examined for at least 20 minutes for a thin smear and 5 minutes for a thick smear. Parasites found were classified as to species and stage, but were not counted. The parasite rates by months for the three stations are given in Table XII. In this table the rates given for all stations are for children from 0-9 years of age. Both sexes were included in all rates.

TABLE XII.

*Parasite rates of children 0-9 years of age (by months and years in three stations).*

Months.	NAGENHALLI.		MUDIGERE		HIRIYUR.	
	1928	1929	January 1930 and Decem- ber 1928.	1929.	1929	1930.
January ..	..	29.6±4.2	25.0±4.4	18.7±3.8	12.5±4.5	20.0±7.0
February ..	..	34.7±4.5	..	23.1±0.2	20.5±4.4	48.9±5.0
March ..	..	39.0±5.1	..	41.5±3.7	35.5±5.8	56.0±6.7
April ..	..	70.6±5.2	..	29.3±3.5	35.6±5.1	53.1±5.9
May ..	..	57.5±5.2	..	46.7±3.3	52.9±8.2	38.9±5.5
June ..	..	35.0±5.1	..	45.8±4.8	52.0±6.7	38.9±7.7
July ..	..	40.0±7.4	..	48.6±5.7	..	36.4±9.8
August ..	..	31.0±4.8	..	34.1±4.7	50.0±23.8	..
September ..	..	32.1±5.9	..	24.3±4.7	25.0±4.0	33.3±6.9
October ..	31.7±3.5	30.4±6.5	..	25.7±5.0	35.4±4.7	..
November ..	46.5±5.1	34.5±5.9	..	20.2±3.0	40.0±6.7	47.8±4.9
December ..	31.7±4.0	26.3±3.8	27.9±3.7	25.9±3.9	57.1±8.9	16.7±7.2
All months ..	37.5±1.3		31.0±1.0		33.7±1.8	43.1±2.1

The parasite rate for all examinations in the Nagenhalli area was significantly higher than that for the Mudigere area, but was not different from either of the two rates for Hiriya town. The Mudigere area rate was not different from the 1929 Hiriya rate, but was significantly lower than the 1930 rate. The 1930 Hiriya rate was higher than the 1929 rate, the difference

being  $9.4 \pm 2.8$ . It is of interest to note that, although the Hiriyr spleen rates for both years were significantly lower by wide margins than the spleen rates of the other two stations, the parasite rates were not below those of the other areas and in 1930 were above the rate of the Mudigere area.

There was no significant difference between the 1928 and 1929 rates for October, November and December in the Nagenhalli area, nor in the rates for December 1928 and 1929 and January 1929 and 1930 in the Mudigere area. The 0-9 year rates for Hiriyr town were lacking in three months out of the twenty-four, and were not significantly greater than their probable errors in January 1929 and 1930, August 1929, and December 1930.

Taking the same three groupings of months as were used in discussing the spleen rates, Table XIII gives the parasite rates of the three seasons for children 0-9 years of age in the Nagenhalli and Mudigere areas and for all examinations in Hiriyr town. The figures given for the Nagenhalli and Mudigere areas are for 1929 only.

TABLE XIII.  
*Parasite rates by groups of months.*

Groups of months.			NAGENHALLI AREA. 0-9 years.	MUDIGERE AREA. 0-9 years.	HIRIYUR TOWN. All ages.	
			1929. Parasite rates.	1929. Parasite rates.	1929. Parasite rates.	1930. Parasite rates.
F. M. A. M.	I	..	$48.5 \pm 2.6$	$34.0 \pm 1.6$	$37.6 \pm 2.0$	$49.1 \pm 2.2$
J. J. A. S.	II	..	$33.8 \pm 2.8$	$38.3 \pm 2.5$	$38.6 \pm 2.0$	$37.2 \pm 2.5$
O. N. D. J	III	..	$30.4 \pm 2.8$	$22.2 \pm 1.9$	$34.5 \pm 2.1$	$37.7 \pm 2.2$
For year	..	..	$38.6 \pm 1.6$	$31.6 \pm 1.1$	$37.0 \pm 1.2$	$41.8 \pm 1.3$

In the Nagenhalli area the parasite rate for group I of months was significantly higher than the rates for groups II and III, the differences being  $14.7 \pm 3.8$  and  $18.1 \pm 3.8$  respectively. The rates for groups II and III were not different. The parasite rates in this area make it seem probable that the malaria season was between February and May, with about one-third of the people having demonstrable parasites in their blood at other times of the year.

The parasite rates for groups of months I and II in the Mudigere area were not different, but both rates were significantly higher than the rate for group III, the differences being  $11.8 \pm 2.5$  and  $16.1 \pm 3.1$  respectively.

The malaria season in this area would seem to be between February and September, with about one-quarter of the population having parasites in their blood during the winter months.

For 1929, the Hiriur parasite rates gave no significant differences among the three groups of months, but in 1930 the rate for group I was significantly higher than for the other two groups, the differences being  $11.9 \pm 3.3$  and  $11.4 \pm 3.1$  respectively. The 1930 rate for group I was significantly higher than the 1929 rate, but this was the only difference of significance between the two years. In the Hiriur area the parasite rates gave no definite indication of any malaria season, what evidence there was somewhat favouring the February to May period. Something over a third of the people had parasites in their blood at all times of the year.

#### SPECIES OF PARASITES.

As was mentioned above, a record was kept of the diagnosis of species of parasite found in all blood slides examined. There are undoubtedly differences in diagnoses of species of parasite between different examiners. It seems, however, that the various examiners of the slides here represented were in substantial agreement, since the results recorded were not very much affected by frequent transfer of staff or visits of inspection by supervising officers. The parasite rates and percentages of all infections found for each species and for mixed infections are given in Table XIV. All rates and percentages are for children of both sexes, and of 0-9 years of age. Since neither the parasite rates nor the percentages of all infections were significantly different

TABLE XIV.

*Parasite rates and percentages of all infections by species of parasites in all examinations of children 0-9 years old in three stations.*

	NAGENHALLI.		MUDIGERE		HIRIYUR.	
	Parasite rate.	Per cent of all infections.	Parasite rate.	Per cent of all infections.	Parasite rate.	Per cent of all infections.
Benign tertian	$16.9 \pm 1.0$	$45.0 \pm 2.2$	$24.7 \pm 1.0$	$79.9 \pm 1.6$	$6.0 \pm 0.7$	$15.9 \pm 1.6$
Malignant tertian	$4.3 \pm 0.6$	$11.4 \pm 1.4$	$1.5 \pm 0.3$	$4.9 \pm 0.9$	$8.6 \pm 0.8$	$22.7 \pm 1.9$
Quartan	$15.1 \pm 1.0$	$40.2 \pm 2.1$	$4.4 \pm 0.4$	$13.8 \pm 1.4$	$21.8 \pm 1.1$	$57.3 \pm 2.2$
Mixed infections	$1.2 \pm 0.3$	$3.4 \pm 0.8$	$0.4 \pm 0.1$	$1.4 \pm 0.5$	$1.6 \pm 0.3$	$4.1 \pm 0.9$
All infections	$37.5 \pm 1.3$	100.0	$31.0 \pm 0.1$	100.0	$38.0 \pm 1.3$	100.0

in Hiriyr town in 1929 from those found in 1930, the examinations for these two years were combined.

Considering first the columns of Table XIV, it is found that both the actual and relative occurrence of the three species of parasites were significantly different from each other in the Mudigere area, but that in Nagenhalli, benign, tertian and quartan were not different from each other in either rate or percentage. In the Hiriyr town the species parasite rates and percentages of benign tertian and malignant tertian were not significantly different, but those for quartan were higher than for either of the other two species.

Considering the rows of this table it is found that the species parasite rate and percentage of all infections for each species were significantly different in each station from corresponding rates in the other two stations. This was not true in the rates and percentages of mixed infections which were in no case different.

When the species of parasites represented in the mixed infections were classified it could be said that the three species occurred in the following rates, ratios on the basis of 100, in the three stations.

Nagenhalli area	..	B. T.—M. T.—Q.	=	46—11—43
Mudigere area	..	B. T.—M. T.—Q.	=	81—5—14
Hiriyr area	..	B. T.—M. T.—Q.	=	17—24—59

The species parasite rates for the three groups of months already used were figured and are given in Table XV. The rates are based on examinations of children 0-9 years of age in the Nagenhalli and Mudigere areas, and on all examinations in 1929 and 1930 in Hiriyr town.

In the Nagenhalli area the benign tertian parasite rates for groups of months I and III were equivalent, but both were significantly higher than the rate for group II. The benign tertian rates in the Mudigere area for groups I and II were equivalent and both were higher than the rate for group III. In Hiriyr during 1929, the rate for group I was higher than that for group II and equivalent to the group III rate, but the rates for groups II and III were not significantly different, while in 1930 the three rates were equal. It seems probable that, in the Nagenhalli area and in Hiriyr, the benign tertian season started in October and lasted through to June, while in Mudigere it started about February and died out in September.

The malignant tertian parasite rate in the Nagenhalli area was highest in group I, but the rates for the three groups were not significantly different from each other in view of their probable errors. In the Mudigere area the rate for group I was the only one of the three which could be regarded as significant. The malignant tertian rates for groups I and II were equal in Hiriyr during 1929 and both were significantly higher than the group III rate, whereas in 1930 the three Hiriyr rates did not differ. Evidence in regard to a possible malignant tertian season was confused, but it seemed probable from the results





here recorded that malignant tertian favoured the first six months of the year rather than the later months.

The occurrence of the quartan parasite was not different in the three groups of months in the Nagenhalli area and in Hiriya during 1929. In Mudigere the highest rate was in group III but this rate was not significantly different from the lower group I rate while the rate for group II was not of use. In Hiriya, 1930, the rate for group I was significantly higher than the rates for the other two groups. Here again the evidence as to a season was confused. It seemed possible, however, that the quartan season, in the Mudigere area at least, started in October, but that in Hiriya it sometimes reached a maximum between February and May.

#### GAMETOCYTE RATES.

Out of 1,337 blood slides in which malaria parasites were found, but 174, or 13.0 per cent, were stated to have shown parasites in the gametocyte stage. This was a very low gametocyte rate for which there is as yet no explanation. It is probably true that many gametocytes were missed and others not correctly diagnosed, but frequent checks by the officer-in-charge did not succeed in raising the rates to any extent. The gametocyte rates for benign tertian and quartan infections seemed noticeably too low. Table XVI gives the gametocyte rates for all examinations made in the three stations.

TABLE XVI.

*Gametocyte rates. All stations. All examinations.*

	Benign tertian.	Malignant tertian.	Quartan.	All species.
Number of slides showing parasites	610	219	508	1,337
Number showing gametocytes.	56	28	90	174
Gametocyte rates ..	9.2±0.8	12.8±1.5	17.7±1.1	13.0±0.6

When the occurrence of gametocyte diagnoses was divided into the three groups of months used above, no significant differences in the rates could be demonstrated in any of the species of parasites. As far as this study went, the gametocyte rates did not show seasonal variations.

#### RELATIONS OF PARASITE RATES.

Certain data in regard to rainfall, average mean, maximum and minimum temperatures, average 8 a.m. relative humidities, parasite rates and spleen rates have been given. It was not found possible to combine the data for the three

stations in order to demonstrate possible relations, due to variations in such relations in the three stations. It was, therefore, necessary to try to correlate the figures for the three stations separately. This meant a maximum of 15 pairs of observations for Nagenhalli, 14 for Mudigere and 24 for Hiriyur—very small numbers of observations on which to draw conclusions. It was felt however that, with proper precautions as to the significance of the coefficients of correlation, some points of value might be demonstrated. For the correlations attempted, the parasite and spleen rates of the 0-9 year group at Mudigere and Nagenhalli stations were used, and the rates for all ages at Hiriyur station, so that all the rates used were significantly greater than their probable errors. It was not possible to avoid the use of rates for monthly figures, since the numbers of people examined varied for each month and the actual numbers of infected had little significance. All conclusions drawn relate to the rates as such.

The correlation tables were first made by grouping. It was later felt that grouping such small numbers of observations might result in false coefficients, so several of the coefficients were obtained again without grouping. For the coefficients so tested grouping made no appreciable difference in the result.

The question as to whether the data used were normally distributed must also be considered. Some of the material was undoubtedly in a skew distribution of greater or lesser degree, but it was felt that the use of 'eta' with such small numbers of observations would be even more questionable and less possible of confirmation by significance tests than the use of 'r' in somewhat skew distributions. The rainfall figures were the most skew.

Since there were so many possibilities of error, it was not thought worth while to go into partial correlations, and, since the relations varied for each place tested, it was obvious that regression equations would be of no value for general application. The remarks here made will also apply to the coefficients of correlation to be given at the end of Part II of this report.

Table XVII gives certain of the coefficients of correlation obtained. Since the probable errors of 'r' are of no use in judging significance where such small numbers are concerned, they have not been given. There was no significant correlation between the parasite rates and the minimum or maximum temperatures (nor was any relation of these two temperatures demonstrable for the data used in Part II), so these correlations have been omitted.

Considering the three groups of months used previously, the one significant coefficient of correlation for the Hiriyur station did not add much to the information already obtained. In Mudigere the significant correlation tended to confirm previous statements that group II was at least a part of the malaria season. In Nagenhalli the highest parasite rates followed one month after the time of highest mean temperatures, so serving to help eliminate group II from consideration as a malaria season. Further discussion of these coefficients will be left until those to be given at the end of Part II of this report are discussed.

TABLE XVII.

*Coefficients of correlation with parasite rates in three stations.*

	Nagenhalli.	Mudigere.	Hiriyur.
Parasite rates and spleen rates ..	+ 0'15	+ 0'48	* + 0'52
Parasite rates and rainfall .. ..	+ 0'50	* + 0'67	— 0'06
Parasite rates and mean temperatures (1 month lag of rates).	* + 0'76	+ 0'25	+ 0'11
Parasite rates and humidity (2 months lag of rates).	— 0'49	— 0'43	— 0'45

\* These coefficients were more than three times greater than the probable error resulting from letting  $r = 0$ . The probability of their arising by random sampling from uncorrelated data was in each case less than 0'01. They may be regarded as significant. The remaining coefficients are not significant.

### SUMMARY.

In this first part of the notes on malaria in Mysore State, South India, a description is given of three stations selected for intensive study. The first station, Nagenhalli, is in an irrigated area and has a moderate rainfall; the second station, Mudigere, has a heavy rainfall; the third, Hiriyur, is in a semi-irrigated area with a low rainfall. At these stations daily records were kept of maximum and minimum temperatures and 8 a.m. relative humidities. Parasite and spleen rates were determined once a month and anophelines were caught in definite stations bi-weekly.

Since little was known of the malaria season in the State, Part I deals with the available data with a view to discovering a season if possible. For this purpose the year was divided into three groups as follows: Group I—February, March, April and May, hot and dry. Group II—June, July, August and September, cooler, and with the highest rainfall. Group III—October, November, December and January, cool and with moderate rainfall. The evidence adduced as to a malaria season may be summarized as follows for each station:

*Nagenhalli.*—There was no dispensary here and deaths from fevers gave no information. The evidence pointed to a season in the months of group I, with a possible beginning in group III. The most that can be said is that in group II there was no apparent spread of malaria. This applied also to the benign tertian parasite; there was not sufficient evidence to show the seasonal differences in the other two species. The relative frequency of the occurrence of the three parasites, benign tertian, malignant tertian, quartan was as 46—11—43.

*Mudigere.*—All the evidence pointed to groups I and II being the important months here. The quartan parasite apparently started its season in group III

and died out in group II. The relative frequency of the occurrence of the three parasites was 81—5—14.

*Hiriyur*.—There was some change in seasonal distribution here during the two years of study. Considering the two years together it seemed probable that group I was a definite season, with a possibility of beginning transmission of malaria in group III. Here, as in Nagenhalli, group II could be definitely ruled out. The relative frequency of the occurrence of the three parasites, benign tertian, malignant tertian, quartan was as 17—24—59.

A consideration of the anophelines of Mysore and their relation to malaria has been reserved for Part II of this report.



## NOTES ON MALARIA IN MYSORE STATE.\*

### Part II.

#### THE ANOPHELINES OF MYSORE STATE.

BY

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IN Part I of this report a description was given of three stations chosen for intensive study of malaria as it exists in Mysore State. These three stations are as follows :—

1. The Nagenhalli area in Mysore District, three miles from Mysore City.
2. Mudigere Town area in Kadur District.
3. Hiriyur Town area in Chitaldrug District.

Work began in the first of these areas on 1st October, 1928, in the second in December, 1928, and in the third in January, 1929. Since those dates the following anophelines have been identified from each station. Only species identified as adults have been included in the list, and specimens have been deposited in the museum of the Malaria Survey of India.

#### 1. Nagenhalli area.

† <i>A. aconitus</i> Dönitz, 1902.	<i>A. hyrcanus</i> var. <i>nigerrimus</i> Giles, 1900.
<i>A. barbirostris</i> van der Wulp, 1884.	<i>A. jamesii</i> Theobald, 1901.
<i>A. culicifacies</i> Giles, 1901.	<i>A. jeyporiensis</i> James, 1902.
<i>A. fuliginosus</i> Giles, 1900.	<i>A. listoni</i> Liston, 1901.

\* The work here reported was done with the support and under the auspices of the Government of Mysore and the International Health Division of the Rockefeller Foundation.

† The following changes in nomenclature have now been adopted by the Malaria Survey of India, in view of the recent researches of Christophers and Edwards:—

Correct name.	Synonym.
(i) <i>A. annularis</i> van der Wulp, 1884.	<i>A. fuliginosus</i> Giles, 1900.
(ii) <i>A. splendidus</i> Koidsumi, 1920.	<i>A. maculipalpis</i> var. <i>indiensis</i> Theobald, 1903.
(iii) <i>A. fluviatilis</i> James, 1902	<i>A. listoni</i> Liston, 1901

*A. maculipalpis* var. *indiensis*  
Theobald, 1903.  
*A. pallidus* Theobald, 1901.  
*A. philippinensis* Ludlow, 1902.

*A. stephensi* Liston, 1901.  
*A. subpictus* Grassi, 1899.  
*A. tessellatus* Theobald, 1901.  
*A. turkhudi* Liston, 1901.

*A. vagus* Dönitz, 1902.

## 2. Mudigere area.

*A. aconitus*.  
*A. aitkenii* James, 1903.  
*A. barbirostris*.  
*A. culicifacies*.  
*A. fuliginosus*.  
*A. hyrcanus* var. *nigerrimus*.  
*A. jamesii*.  
*A. jeyporiensis*.  
*A. karwari* James, 1903.  
*A. leucosphyrus* Dönitz, 1901.  
*A. listoni*.

*A. maculatus* Theobald, 1901.  
*A. maculipalpis* var. *indiensis*.  
*A. majidi* Young and Majid, 1928.  
*A. minimus* Theobald, 1901.  
*A. pallidus*.  
*A. philippinensis*.  
*A. subpictus*.  
*A. tessellatus*.  
*A. turkhudi*.  
*A. vagus*.  
*A. varuna* Iyengar, 1924.

## 3. Hiriyur area.

*A. aconitus*.  
*A. barbirostris*.  
*A. culicifacies*.  
*A. fuliginosus*.  
*A. hyrcanus* var. *nigerrimus*.  
*A. jamesii*.  
*A. jeyporiensis*.  
*A. listoni*.  
*A. maculipalpis* var. *indiensis*.

*A. minimus*.  
*A. pallidus*.  
*A. philippinensis*.  
*A. stephensi*.  
*A. subpictus*.  
*A. tessellatus*.  
*A. turkhudi*.  
*A. vagus*.  
*A. varuna*.

Of the 22 species here noted, thirteen had been reported from various parts of the State previous to 1928. A larva identified as *A. insulae-florum* was caught in the Mudigere area but as it failed to develop further it was not included in the list. Since then, similar larvæ caught in the western part of the Hassan District developed into adults and were identified as *A. insulae-florum*. This species might, consequently, be included in the report of the anophelines of Mysore.

## SEASONAL OCCURRENCE OF ANOPHELINES.

From the catches of larval and adult anophelines in the three stations (during 1928 and 1929 in the Nagenhalli area, 1929 in the Mudigere area, and 1929 and 1930 in the Hiriyur area), it was possible to draw conclusions as to the seasonal occurrence of the anopheline species in Mysore. The same three groups of months as those used in Part I of this report were used for this purpose. Table I shows the maximum seasonal prevalence of the species caught

in the three stations, this season being determined on the basis of both adult and larval catches. Where blanks are left in the table, either the catches were too small to allow of any conclusions or the species was not caught.

TABLE I.

*Maximum occurrence of anopheline species by the three groups of months\* in three study stations.*

Species of anophelines.	Nagenhalli area.	Mudigere area.	Hiriyur area.
<i>aconitus</i> .. ..	III	..	III
<i>aithensii</i> .. ..	..	I	..
<i>barbirostris</i> .. ..	III	III	III
<i>culicifacies</i> .. ..	II	I	II and III
<i>fuliginosus</i> .. ..	I	I	II
<i>hyrcanus</i> .. ..	III	III	..
<i>jamesii</i> .. ..	III	II	..
<i>jeyporiensis</i> .. ..	III	I and II	I and III
<i>karwari</i> .. ..	..	II and III	..
<i>leucosphyrus</i> .. ..	..	I and II	..
<i>listonii</i> † .. ..	II and III	I	III
<i>maculatus</i> .. ..	..	I	..
<i>maculipalpis</i> var. <i>indiensis</i> .. ..	II	I	III
<i>majidi</i> .. ..	..	I	..
<i>pallidus</i> .. ..	I	I	I
<i>philippinensis</i> .. ..	II	II	..
<i>stephensi</i> .. ..	I	..	III
<i>subpictus</i> .. ..	I and II	..	I
<i>tessellatus</i> .. ..	II	III	II and III
<i>turkhudi</i> .. ..	I	..	I
<i>vagus</i> .. ..	II and III	I	I and II

\* Group I. February, March, April, May.

Group II. June, July, August, September.

Group III. October, November, December, January.

† Under *listonii* are included *minimus* and *varuna*, as at the time of these collections the three species were not adequately distinguished.

With the exception of nine species, the seasonal occurrence of the species of anophelines was in agreement in the three stations. As far as could be determined from the data available, the differences were real in the case of *culicifacies*, *fuliginosus*, *jamesii*, *jeyporiensis*, *listonii* and *vagus*. *A. maculipalpis* var. *indiensis* was rarely caught in any station; *stephensi* was rare in Nagenhalli and absent in Mudigere; *tessellatus* was rarely caught in Nagenhalli and Mudigere. It is of special interest to note that both *culicifacies* and the *listonii* groups had their maximum occurrence in the Mudigere area in the months of group I, in contrast to their occurrence in the other two stations. The breeding habits of the species reported were not essentially different from those recorded in other parts of India, so they have not been discussed again.



### RESULTS OF DISSECTIONS OF ANOPHELINES.

In each of the three study areas certain houses and cattle-sheds, and combinations of them, were selected as catching stations. These were visited twice a week and adult anophelines caught. Anophelines captured were preserved in lamp chimneys and all females were dissected, sometimes after 60 hours but more often after 72 hours. A report published in 1931 gave the results of the dissections done previous to 31st December, 1930 (Sweet and Rao, 1931). In that report dissections of 31,277 mosquitoes were listed, of which eleven specimens were found infected. Three of these were gut infections and eight were gland infections. The species found infected were *A. culicifacies*, the *listonii* group (*A. listonii*, *A. minimus* or *A. varuna*), and *A. stephensi*. Since January, 1931 dissections have been made at various times of the year in both epidemic and endemic areas. The report of these additional dissections will be deferred until the data are more complete, but it may be stated here that no other species of anophelines have been found infected. It seems fair to assume, under these circumstances, that the dangerous species of anophelines in Mysore State, as far as malaria is concerned, are *A. culicifacies*, *A. stephensi* and either one, two or all of the *listonii* group (*A. listonii*, *A. minimus* and *A. varuna*).

Of the eleven infected mosquitoes previously reported, six were found in the regular study areas and five during epidemics in various other parts of the State. Infected anophelines were reported from the Nagenhalli area in April (3) and August (1); from the Hiriya area in September (2). Since that report, further infections have been found in the Nagenhalli area in March and April, the Mudigere area in May and the Hiriya area in December. Infected mosquitoes reported from epidemic areas have been found in June, August, October and November.

### OCCURRENCE OF DANGEROUS ANOPHELINES.

Before control work began, the catches of females of the dangerous anopheline species in the selected catching stations of the Nagenhalli area were 5,013; in the Mudigere area, 325; in the Hiriya area (two full years), 18,712. The incidence of these catches by species, and by the three groups of months previously used, is given in Table II. In the Nagenhalli area just under half of the total catch of females of the dangerous species was in the months of group II, and just over a quarter in the months of group III. About 65 per cent of the total catch of females of these species in the Mudigere area was in the months of group I, while in the Hiriya area 57 per cent of the catch was in the months of group III and one-third in the months of group II.

Of the catching stations selected in the three study areas, the great majority were combined dwellings and cattle-sheds, due to the living habits of the people of the State. It was not possible, in such stations, to separate the catches with any degree of assumed accuracy. However, a few of the stations selected could be classified as more or less pure dwellings, and a few more as

TABLE II.

*Seasonal occurrence of catches of females of the dangerous species of anophelines.*

Species of <i>anopheles</i> .	Groups of months.	NAGENHALLI AREA.		MUDIGERE AREA.		HIRIYUR AREA.	
		No.	Per cent.	No.	Per cent.	No.	Per cent.
<i>A. culicifacies</i> ..	I	913	27.2±0.5	90	73.2±2.7	619	9.4±0.2
	II	1909	56.9±0.6	17	13.8±2.1	2641	40.1±0.4
	III	534	15.9±0.4	16	13.0±2.0	3319	50.5±0.4
<i>listonii</i> group. ( <i>A. listonii</i> , <i>A. minimus</i> , and <i>A. varuna</i> .)	I	249	16.4±0.6	123	60.9±2.3	883	11.1±0.2
	II	476	31.2±0.8	27	13.4±1.7	2114	26.4±0.3
	III	797	52.4±0.9	52	25.7±2.1	4989	62.5±0.4
<i>A. stephensi</i> ..	I	58	43.0±2.8	..	..	217	5.2±0.2
	II	34	25.2±2.5	..	..	1521	36.7±0.5
	III	43	31.8±2.7	..	..	2409	58.1±0.5
All dangerous anophelines.	I	1220	24.3±0.4	213	65.6±1.8	1719	9.2±0.1
	II	2419	48.2±0.5	44	13.5±1.3	6276	33.5±0.2
	III	1374	27.4±0.4	68	20.9±1.5	10717	57.3±0.2

pure cattle-sheds. All the catches made were accordingly classified as to type of source, combined house and cattle-shed, house, and cattle-shed. For the figures in the following discussion, the catches in combined house and cattle-shed stations were not considered. It was not found possible during this study to test for the source of the blood meal in the stomachs of mosquitoes caught. Since this was so, it was thought that the relative and absolute occurrence in house catches of females of the dangerous species, and of other species, might give some slight indications of habits which would possibly prove of interest. Although the many sources of error in such a consideration of data were recognized it was thought worth while to include some discussion of this aspect of the catches.

The figures for catches in pure houses and cattle-sheds were analysed to show the total numbers caught of females of dangerous species, and other species, as well as the percentages of these totals caught in houses. A second analysis was made of the total number of anophelines caught in pure houses, and the percentages of these totals which were of the dangerous species. Tables III, IV and V give these figures, for the three groups of months, in the Nagenhalli, Mudigere and the Hiriya areas. It should be mentioned that the data used in the first of these two analyses were arranged in the form of fourfold tables and tested by chi-square. The parts of the tables were numbers of females of dangerous species caught in (a) houses and (b) cattle-sheds and the same figures for females of other species. Wherever the two corresponding percentages (those of catches of dangerous species and other species) of the first part of Tables III, IV and V are significantly different from each other,

the fourfold chi-square tests were also significant. The details of these tests have not been reproduced here in order to save space.

TABLE III.

*Occurrence of female anophelines of dangerous species in houses of the Nagenhalli area.*

	Months of group I.	Months of group II.	Months of group III.	All months.
Total catch of female anophelines of dangerous species in pure houses and cattle-sheds.	380	459	668	1,507
Percentage of this catch which were caught in houses.	$29 \pm 0.6$	$37 \pm 0.6$	$57 \pm 0.6$	$44 \pm 0.3$
Total catch of female anophelines of other species in pure houses and cattle-sheds.	328	321	784	1,433
Percentage of this catch which were caught in pure houses.	$64 \pm 0.9$	$87 \pm 1.1$	$26 \pm 0.4$	$48 \pm 0.4$
Total catch of female anophelines of all species in pure houses.	32	45	58	135
Percentage of this catch which were of dangerous species.	$34.4 \pm 5.7$	$37.8 \pm 4.9$	$65.5 \pm 4.2$	$48.9 \pm 2.9$

Of a total of 1,507 female anophelines of dangerous species caught in houses and cattle-sheds of the Nagenhalli area,  $4.4 \pm 0.3$  per cent were caught in houses. The corresponding percentage for females of other species of anophelines was  $4.8 \pm 0.4$ . These percentages for all months were not significantly different. The two corresponding percentages for the months of group I were also not significantly different. In the months of group II, however, a significantly greater percentage of the total catch of females of other species was caught in houses than that of females of the dangerous species. This relationship was reversed in the months of group III, when a significantly higher percentage of the total catch of females of the dangerous species was caught in houses than was the case for females of other species. From the second part of the analysis, it was found that  $65.5 \pm 4.2$  per cent of the total house catches during the months of group III were females of the so-called dangerous species, a percentage that was significantly higher than the corresponding percentages for the months of groups I and II. It was probable, then, that only in the months of group III in the Nagenhalli area did females of the dangerous species seem more likely to seek out houses than females of other species, and that in these months something over half of the house catches were of the dangerous species.

TABLE IV.

*Occurrence of female anophelines of the dangerous species in houses of the Mudigere area.*

	Months of group I.	Months of group II.	Months of group III.	All months.
Total catch of female anophelines of dangerous species in pure houses and cattle-sheds.	213	46	70	329
Percentage of this catch which were caught in houses.	$22.5 \pm 1.9$	$45.7 \pm 4.9$	$11.4 \pm 2.6$	$23.4 \pm 1.6$
Total catch of female anophelines of other species in pure houses and cattle-sheds.	3,061	6,592	4,174	13,827
Percentage of this catch which were caught in pure houses.	$12.6 \pm 0.4$	$10.7 \pm 0.3$	$7.8 \pm 0.3$	$10.3 \pm 0.2$
Total catch of female anophelines of all species in pure houses	434	729	332	1,495
Percentage of this catch which were of dangerous species.	$11.1 \pm 1.0$	$2.9 \pm 0.4$	$2.4 \pm 0.6$	$5.1 \pm 0.4$

In the Mudigere area, from the figures of Table IV, it was probable that in the months of groups I and II females of the dangerous species were more

TABLE V.

*Occurrence of female anophelines of dangerous species in houses of the Hiriur area.*

	Months of group I.	Months of group II.	Months of group III.	All months.
Total catch of female anophelines of dangerous species in pure houses and cattle-sheds.	91	624	615	1,330
Percentage of this catch which were caught in houses.	$26.4 \pm 3.1$	$18.1 \pm 1.0$	$22.4 \pm 1.1$	$20.7 \pm 0.7$
Total catch of female anophelines of other species in pure houses and cattle-sheds.	424	287	198	909
Percentage of this catch which were caught in pure houses.	$25.2 \pm 1.4$	$20.2 \pm 1.6$	$30.8 \pm 2.2$	$24.9 \pm 1.0$
Total catch of female anophelines of all species in pure houses.	131	171	199	501
Percentage of this catch which were of dangerous species.	$18.3 \pm 2.3$	$66.1 \pm 2.4$	$69.3 \pm 2.2$	$54.9 \pm 1.4$

likely to be found in houses than were females of other species, and that the highest percentage of house catches of the dangerous species was in the months of group I. Even in these months this percentage was low due to the small total catch of females of the dangerous species.

Considering the figures for the Hiriur area, given in Table V, there did not seem to be any difference in the choice of houses as resting places between females of the dangerous species and of other species. There was some indication, when the figures for all months were considered, that females of other species were slightly more likely to be found in houses. However, in the months of groups II and III well over half of the total house catches were females of the dangerous species.

### RELATIONS OF ANOPHELINE CATCHES.

As has been stated, anophelines were caught at certain selected stations in each of the areas, the catches being on a time basis. For determining, if possible, any relations between these anopheline catches and other factors

TABLE VI.

*Average catch of anophelines per catching station per month in three study areas.*

November 1928 to December 1930 (inclusive).	NAGENHALLI AREA.		MUDIGERE AREA.		HIRIYUR AREA.	
	Average dangerous anophelines.	Average other anophelines.	Average dangerous anophelines.	Average other anophelines.	Average dangerous anophelines.	Average other anophelines.
November ..	40	30	..	30	..	..
December ..	43	24	0.6	..	..	..
January ..	31	13	1.0	25	11	5
February ..	31	16	1.0	32	26	44
March ..	12	21	0.8	23	7	43
April ..	13	37	3.0	21	6	45
May ..	19	32	3.0	32	6	40
June ..	14	12	1.0	25	7	16
July ..	30	22	0.3	57	31	9
August ..	61	71	0.1	93	104	52
September ..	36	58	0.0	60	73	33
October ..	24	26	0.0	33	34	37
November ..	44	33	0.4	60	78	27
December ..	41	20	0.6	30	72	7
January ..	32	24	1.8	18	15	11
February ..	..	..	..	..	9	26
March ..	..	..	..	..	6	18
April ..	..	..	..	..	4	13
May ..	..	..	..	..	4	9
June ..	..	..	..	..	13	11
July ..	..	..	..	..	27	5
August ..	..	..	..	..	26	14
September ..	..	..	..	..	29	18
October ..	..	..	..	..	21	36
November ..	..	..	..	..	128	49
December ..	..	..	..	..	74	16

studied, it was thought best to use the average catch per station per month. The figures for these average catches, for anophelines of the dangerous species and for anophelines of other species, are given in Table VI.

Under the section on 'Relations of Parasite Rates', in Part I of this report, certain remarks were made concerning the correlations set up on the data available. It was stated that those remarks apply also to the correlations of this part of the report, they will therefore not be repeated here. Table VII gives the coefficients of correlation for those correlations found to be of interest in connection with the anopheline data. As in Part I the probable errors of the coefficients were omitted, as they were of little value in judging significance due to the small number of pairs of observations.

TABLE VII.

*Certain of the coefficients of correlation obtained with the data on average catch of anophelines per catching station per month.*

Correlations between	Nagenhalli area.	Mudigere area.	Hiriyur area.
Average catch of dangerous anophelines and average catch of other anophelines.	+ 0.51	- 0.52	+ 0.36
Average catch of other anophelines and mean temperatures.	- 0.09	- 0.69 *	- 0.09
Average catch of dangerous anophelines and mean temperatures.	- 0.36	+ 0.58	- 0.57 *
Average catch of other anophelines and average 8 a.m. humidity (one month lag of average catch).	- 0.27	+ 0.75 *	+ 0.33
Average catch of dangerous anophelines and average 8 a.m. humidity (one month lag of average catch).	+ 0.62 †	- 0.56	+ 0.64 *
Average catch of other anophelines and parasite rates (one month lag of parasite rates).	- 0.11	- 0.35	- 0.09
Average catch of dangerous anophelines and parasite rates (one month lag of parasite rates).	- 0.69 *	+ 0.72 *	- 0.49 †
Average catch of <i>A. culicifacies</i> and parasite rates (one month lag of parasite rates).	- 0.08	+ 0.67 †	- 0.64 †
Average catch of anophelines of <i>listoni</i> group and parasite rates (one month lag of parasite rates).	- 0.59	+ 0.71 *	- 0.40
Average catch of <i>A. stephensi</i> and parasite rates (one month lag of parasite rates).	+ 0.05	.	- 0.41
Average catch of <i>A. culicifacies</i> and <i>listoni</i> group and parasite rates (one month lag of parasite rates).	- 0.67 †	..	..

\* These coefficients were more than three times greater than the probable error resulting from letting  $r=0$ . The probability of their arising by random sampling from uncorrelated data was in each case less than 0.01. They may be regarded as significant.

† These coefficients fulfilled the same tests, except that their probabilities were between 0.01 and 0.02. The remaining coefficients were not regarded as significant by these two tests.

The average catches of anophelines of dangerous and other species were not significantly correlated in any station. The coefficients were positive in sign in the Nagenhalli and Hiriya areas but negative in the Mudigere area. In the former two areas the coefficients of the correlations, between average catches of dangerous and other anophelines and the monthly parasite rates, were all negative in sign, whereas in the Mudigere area one was positive and the other negative. Judged by these three sets of correlations, the signs of all the correlations of Table VII above, and of Table XVII of Part I, were correct with the exception of one of the Nagenhalli area correlations. The correlation coefficient in this area, between the average catch of other anophelines and the average 8 a.m. humidity, had a negative sign, whereas it should have been positive in sign to agree with the signs of the other correlations. No explanation was found for this discrepancy.

In the Mudigere area there was a definite negative association between the average catch per station per month of anophelines of the species not considered dangerous and the average monthly mean temperatures. With a one month lag of the average catches, there was a definite positive association of these catches with the average 8 a.m. relative humidities in that area. The same correlations did not yield significant coefficients in the other two areas. In no area were the correlations significant between the average catches of non-dangerous anophelines and the monthly parasite rates.

The average catch per station per month of anophelines, considered as possible malaria carriers, had a significant negative correlation with the mean temperature in the Hiriya area, and a positive correlation with the average 8 a.m. humidity in the Nagenhalli and Hiriya areas. Other correlations of the same kinds in the three areas were not significant.

There was no significant direct correlation between the catch of dangerous anophelines and the parasite rates in any area. When an attempt was made to allow for the incubation period of malaria, both in the mosquito and in man, by introducing a month's lag of the parasite rates in the correlations, the coefficients were all found to be significant. That is, the parasite rate of February was paired with the average catch of January, and so on through the monthly figures available. This significant correlation was positive in sign in the Mudigere area and negative in sign in the Nagenhalli and Hiriya areas.

As was stated previously, the anophelines of the dangerous species were considered to be *A. culicifacies*, the *listonii* group (*listonii*, *minimus* and *varuna*), and *A. stephensi*. In the Nagenhalli figures there was a definite negative correlation between the parasite rates, with a lag of one month, and the average catch per station per month of *culicifacies* plus *listonii* group, but not between either set of catches alone, and the parasite rates. The average catch of *stephensi* did not correlate with the parasite rates and gave a positive coefficient. For the Mudigere area both the average catches of *culicifacies* and of the *listonii* group were significantly correlated with the parasite rates; *stephensi* was not caught in this area. The only significant correlation with the parasite

rates of the Hiriyr area was that with *culicifacies*, the coefficient being negative in sign.

If these correlations express a true relationship, the higher parasite rates in the Nagenhalli and Hiriyr areas may be expected in the months with the lower humidities and higher mean temperatures and with the lower catches of dangerous anophelines. In the Nagenhalli area the relationship will be with catches of both *culicifacies* and mosquitoes of the *listonii* group, while in Hiriyr it will be with *culicifacies* only. In the Mudigere area the higher parasite rates will be in the months with the higher rainfalls, and with the higher catches of *culicifacies* and the *listonii* group.\*

The average mean temperature for the Nagenhalli area, of the months of group I (February, March, April and May), was 80 and the humidity was 70 per cent while but 24 per cent of the total catch of *culicifacies* and *listonii* group were caught in these months. During the months of group III (October, November, December and January), the average mean temperature was 76°, the humidity 79 per cent (the highest of the three groups of months), and the per cent of the total catch was 27. It was, however, during the months of group III that females of the dangerous species were more likely to be caught in dwellings than females of other species, and in these months that over sixty per cent of the catches in dwellings were of the dangerous species. To date, all but one of the infected mosquitoes reported from this area were found in the months of group I. The summary of the data given in Part I stated that the evidence pointed to a malaria season in the months of group III. Most of the evidence from the data on the anophelines would seem to favour the months of group I. It was still not possible, however, to rule out the months of group III as not requiring control work.

In the Hiriyr area, the evidence from the correlations pointed to the months of group I as a malaria season, since these were the months of higher mean temperature, lower humidity and smaller catch of *culicifacies*. As against this there was no evidence that the dangerous anophelines favoured dwellings during these months, and the highest percentage of house catches were dangerous anophelines in the months of groups II and III. Also, reports of infected anophelines from this area were in the months of September and December. Considering the findings of Part I, the conclusion as to a malaria season for this area must be the same as for the Nagenhalli area.

As regards Mudigere, the months of group I yielded about 65 per cent of the total catch of dangerous anophelines and the months of group II had much the higher average rainfall. Females of the dangerous species were more likely to shelter in dwellings during the months of groups I and II than were other females, and the highest percentages of catches in dwellings were of the dangerous species during the months of group I. Infected mosquitoes were

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\* See Table XVII, Part I, for certain of the correlations upon which these statements are based.



reported in May. There seemed to be no question, in agreement with Part I, but that the malaria season in this area was within the first seven months of the year.

### SUMMARY.

Part II of this report of a study of malaria in Mysore State, South India, deals with the anophelines. During the course of the study 22 species of anophelines were identified, of which 13 had been previously recorded. Based on catches of both larvæ and adults a table was given reporting the seasonal occurrence of these species.

A previous report had given the results of dissections of 31,277 mosquitoes, of which eleven were found to be infected. Since that report further dissections had been made but the report of the results of these additional dissections was deferred. To date infections had been reported in *A. culicifacies*, members of the *listonii* group (*A. listonii*, *A. minimus* or *A. varuna*), and *A. stephensi*. These were considered the dangerous anophelines in Mysore. Infected mosquitoes were reported in the months of March, April, May, June, August, September, October, November and December from various parts of the State under either endemic or epidemic conditions.

A table is given showing the percentage of dangerous anophelines caught during three groups of months in each station. The figures show a variation of the occurrence of these species between the Mudigere area and the other two areas. The catches were highest in February, March, April and May (group I) in the Mudigere area; in June, July, August and September (group II) in the Nagenhalli area; and in October, November, December and January (group III) in the Hiriyur area. In the Nagenhalli area females of the dangerous species of anophelines were more likely to be caught in pure dwelling houses than were females of other species, only in the months of group III. During these months over 60 per cent of the dwelling-house catches were of the dangerous species. The same data for the Mudigere area implicated the months of groups I and II. In the Hiriyur area there was no difference in the apparent fondness for houses between the dangerous and other species of anophelines, but well over 60 per cent of the house catches were of the dangerous species in the months of groups II and III.

Coefficients of correlation obtained from the data given in Parts I and II of this report indicated that the higher parasite rates of the Nagenhalli and Hiriyur area were likely to be in, or just after, the months with the lower humidities, higher mean temperatures and lower catches of dangerous anophelines. In the Mudigere area the higher parasite rates were associated with the higher rainfalls and the higher catches of anophelines of the dangerous species. The parasite rates were not associated with catches of anophelines of other than the dangerous species in any station.

### REFERENCE.

SWEET, W. C., and Rao, B. A. (1931). *Rec. Mal. Surv. Ind.*, 2, 4, pp. 655-657.

## NOTES ON MALARIA IN MYSORE STATE.\*

### Part III.

#### SPLEEN AND PARASITE RATE RELATIONSHIPS.

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### INTRODUCTION.

IN Part I of this report parasite and spleen rates were considered mainly in their relations to the time factor. The relations of these rates to other factors were left for consideration in this part of the report. For the purpose a total of 4,345 examinations, made during the last three months of 1928 and during the twelve months of 1929, were analysed. As was stated in Part I, examinations for determining parasite and spleen rates were made monthly in each of three study stations. Each month's work was considered as a new sample with no regard paid to previous examinations. Each time an effort was made to have the sample representative of the population, but it must be remembered that no regimentation of the people was possible, and that examinations were voluntary, as influenced by the persuasion of the staff concerned. Due to this there were a considerable number of re-examinations of the same people in successive months. The 4,345 examinations here reported were not therefore of that number of different persons, but were examinations of a smaller number of people seen at varying intervals over the course of fifteen months.

Of this total number, 3,460 were examinations of males and 885 of females. Further classifications were made by age groups 0-4, 5-9, 10-14, 15-19, and 20 years and over. Of the lowest age group very few examinations were of

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\*The work here reported was done with the support and under the auspices of the Government of Mysore and the International Health Division of the Rockefeller Foundation.

infants one year old or younger, so for all practical purposes that age group was really one of from two to four years.

### RELATIONSHIP OF AGE AND SEX TO PARASITE RATES.

Table I gives the parasite rates as influenced by age and sex.

TABLE I.  
*Infections with malaria parasites by age and sex.*

Age group.	MALES.			FEMALES.			BOTH SEXES.	
	No. exam.	No. inf.	Per cent. inf.	No. exam.	No. inf.	Per cent. inf.	No. exam.	Per cent. inf.
0-4 ..	256	98	38.3±2.0	192	76	39.6±2.4	448	38.8±1.6
5-9 ..	1,268	433	34.1±0.9	434	130	30.0±1.5	1,702	33.1±0.8
0-9 ..	1,524	531	34.8±0.8	626	206	32.9±1.3	2,150	34.3±0.7
10-14 ..	1,096	305	27.8±0.9	136	38	27.9±2.7	1,232	27.8±0.9
15-19 ..	241	72	29.9±2.0	18	2	11.1±5.0	259	28.6±1.9
10-19 ..	1,337	377	28.2±0.8	154	40	26.0±2.4	1,491	28.0±0.8
20 and over	599	146	24.4±1.2	105	12	11.4±2.1	704	22.4±1.1
All ages ..	3,460	1,054	30.5±0.5	885	258	29.2±1.0	4,345	30.2±0.5

The parasite rate of  $11.1 \pm 5.0$  for females between 15 and 19 years of age was not significantly greater than its probable error, and could not be used in comparisons. For the age groups 0-4 and 5-9, the former was possibly higher than the latter for both sexes and for females, but was not higher in examinations of males. The rate for the 0-4 year group was higher than that for the 10-14 year group in examinations of both sexes and of males and females separately. This was also true of the rate for adults, which was decidedly lower than that for the 0-4 year group. The parasite rates for the 5-9 year group were higher than those for the 10-14 year group (not true for females) and than those for the 20 years and over group, but were not significantly different from the rates for those of 15-19 years of age. There was no difference between the significant rates for those 10-14 years old and those 15-19, but the former were higher than the adult rates except in the case of males. Rates for the 15-19 year age group did not differ from those for 20 years and over. In the case of the larger age groups, the 0-9 year rates were higher than those for the 10-19 group (not true for females), and higher than those for adults, while the 10-19 year rates were also higher than the adult rates except in the case of males.

In general it may be stated that (a) there was a distinct lowering of parasite rates with advancing age, and (b) that there was no difference in rates between the two sexes for any age group except in the case of those 15-19 years of age \*, and in the case of those 20 years old and over, where the rate for males was the higher. In view of this difference between the sexes in parasite rates of adults, it seemed possible that the same sex difference may have existed in the case of those 15-19 years of age.

### RELATIONSHIP OF AGE AND SEX TO SPECIES OF PARASITES.

Table II gives the occurrence of the three species of malaria parasites, and of mixed infections, for both sexes by the age groups used in Table I. There was no significant difference between the infection rates of males and females with any species of parasite, nor in infections with more than one species, so separate figures for the sexes are not given.

TABLE II.

*Occurrence of different species of malaria parasite by age groups. (Examinations of both sexes.)†*

Age groups.	BENIGN TERTIAN.		MALIGNANT TERTIAN.		QUARTAN		MULTIPLE INFECTIONS	
	No inf	Per cent.	No. inf	Per cent	No inf	Per cent	No inf	Per cent
0-4 ..	94	21.0±1.3	26	5.8±0.7	49	10.9±1.0	5	1.1±0.3
5-9 ..	277	16.3±0.6	69	4.1±0.3	201	11.8±0.5	16	0.9±0.2
0-9 ..	371	17.3±0.5	95	4.4±0.3	250	11.6±0.5	21	1.0±0.1
10-14 ..	149	12.1±0.6	58	4.7±0.4	127	10.3±0.6	9	0.7±0.2
15-19 ..	30	11.6±1.3	13	5.0±0.9	29	11.2±1.3	2	0.8±0.4
10-19 ..	179	12.0±0.6	71	4.8±0.4	156	10.5±0.5	11	0.7±0.1
20 and over	59	8.4±0.7	35	5.0±0.6	60	8.5±0.7	4	0.6±0.2
All ages ..	609	14.0±0.4	201	4.6±0.2	466	10.7±0.3	36	0.8±0.09

† Numbers of persons examined for each age group the same as in 'Both Sexes' column of Table I

The relations of the benign tertian infection rates to age were practically identical with the relations already noted for the general parasite rates, so they will not be repeated here. The quartan infection rates for the 5-9 year group and the 0-9 year group were both significantly higher than the rates for adults

\* In this group no possible difference could be determined since the rate for females of this age was not significant.

over 20, although other age groups showed no differences. The rate for infections with malignant tertian parasites, and with more than one species of parasite, were all the same. The tendency noted for the general parasite rate to decrease with increasing age was mainly due to the benign tertian infections, with some assistance in this direction from the quartan infections.

To test whether this difference was a significant one, use was made of the chi-square test as demonstrated in Table VI. The distributions tested were as given in Table IIA. The numbers of persons examined were the same for each species of parasite in each age group.

TABLE IIA.

*Occurrence of different species of parasite by age groups.*

	0-4	5-9	10-14	15-19	20 and over.
Benign tertian .. ..	94	277	149	30	59
Malignant tertian .. ..	26	69	58	13	35
Quartan .. ..	49	201	127	29	60

The chi-squares obtained by testing these distributions in pairs were as follows :—

Benign tertian and malignant tertian—chi-square equals ..	14.60
Benign tertian and quartan—chi-square equals ..	9.17
Quartan and malignant tertian—chi-square equals ..	5.57

Of these chi-squares the first two were large enough so that it may be stated that (a) the benign tertian infections diagnosed showed a preference for occurrence in the two younger age groups, when compared with the other two species of parasites, and (b) that there was no difference in this respect between the quartan and malignant tertian infections here reported.

### RELATIONSHIP OF AGE AND SEX TO SPLEEN RATES.

The spleen rates recorded at these examinations did not have the same relations to sex and age as did the parasite rates. Table III gives the spleen rates for the same age groups, by sexes, as those used for Table I. The figures for numbers examined in these two tables do not agree, since certain persons were examined for parasites only. In the examinations of males there was no significant difference in the spleen rates of the various age groups, with the exception of the rates for the 5-9 and 10-14 year groups, where the former rate was possibly higher than the latter. The spleen rates for the females examined did not show any change with advancing age, except between the youngest and oldest age groups, as the 0-4 year rate was probably significantly below that for adults, the same being true for the 0-9 year age group.

TABLE III.

*Percentage of enlarged spleens by age and sex.*

Age groups.	MALES.			FEMALES.			BOTH SEXES.	
	No. exam.	No. with palpable spleen.	Per cent with palpable spleen.	No. exam.	No. positive	Per cent.	No. exam.	Per cent with palpable spleen.
0-4 ..	216	151	69.9±2.1	160	99	61.9±2.6	376	66.5±1.7
5-9 ..	1,262	960	76.1±0.8	426	286	67.1±1.5	1,688	73.8±0.7
0-9 ..	1,478	1,111	75.2±0.7	586	385	65.7±1.3	2,064	72.5±0.6
10-14 ..	1,086	783	72.1±0.9	110	78	70.9±2.9	1,196	72.0±0.9
15-19 ..	237	177	74.7±1.9	7	5	71.4±11.5	244	74.6±1.9
10-19 ..	1,323	960	72.6±0.8	117	83	70.9±2.8	1,440	72.4±0.8
20 and over	584	433	74.1±1.2	9	8	88.9±7.1	593	74.4±1.2
All ages ..	3,385	2,504	74.0±0.5	712	476	66.9±1.2	4,097	72.7±0.5

Considering all examinations of both sexes it was found that (a) the spleen rate of the 0-4 year group was quite definitely lower than that for the 5-9 year group and (b) was probably lower than the rates for the 15-19 year and the 20 years and over groups. There were no other significant differences in spleen rates by age groups.

The spleen rate for males of all ages was  $74.0 \pm 0.5$ , which was significantly higher than the rate of  $66.9 \pm 1.2$  for all females examined. This was also true of the rates for males in the 5-9 and 0-9 year age groups. In general, for these examinations the spleen rates of the youngest age group examined had a tendency to be lower than rates for the older age groups, but aside from this there was no marked change in spleen rates with age. Also, spleen rates for females were below those for males in children under ten, and in all examinations made.

Spleen sizes were recorded by a system once used in the 'Stazione Sperimentale per la Lotta Antimalarica' in Italy. This classification noted seven grades of enlargement relative to the costal margin and the umbilicus, four above the umbilicus and three below. The smallest class was a 'P' spleen felt at the costal margin on inspiration. The area between the costal margin and the umbilicus was divided into three equal parts by visual inspection, and spleens were classified as one, two or three, the latter being around the umbilicus. A similar division was made between the umbilicus, and the lower border of the abdomen, and spleens were classed as four, five or six according to the position of the apex. No spleens in class six were reported during this work. In almost all cases examinations were made with the subject lying down and the knees drawn up.

## RELATIONSHIP BETWEEN PARASITE AND SPLEEN RATES.

Since the relations of the parasite rates to spleen sizes are of interest, Table IV gives these rates by age for both sexes. The parasite rate for those of 0-9 and 10-19 years with spleen not palpable at the time of examination was 17.4 per cent, a rate which was markedly lower than for those of corresponding ages in whom palpable spleens were found. The difference was greatest in the younger age group. This difference was also present in those of all ages, but was not found in the 20 years and over age group. In the latter group the parasite rates were all the same, regardless of presence or absence of a palpable spleen or the size of the enlargement when present.

TABLE IV.

*Parasite rates by spleen size and age. Both sexes.*

	0-9			10-19			20 AND OVER.			ALL AGES.
	No. exam	No inf	Per cent inf.	No exam	No inf	Per cent inf.	No. exam	No inf	Per cent inf.	Per cent inf.
Spleen not palpable	568	99	17.4±1.1	397	69	17.4±1.3	152	37	24.3±2.3	18.3±0.8
Spleen P	297	99	33.3±1.8	216	60	27.8±2.0	87	20	23.0±3.0	29.8±1.3
Spleen 1	477	174	36.5±1.5	339	109	32.2±1.7	132	35	26.5±2.6	33.5±1.0
Spleen 2	450	207	46.0±1.6	322	117	36.3±1.8	127	35	27.6±2.7	39.9±1.1
Spleen 3	234	111	47.4±2.2	139	45	32.4±2.7	78	18	23.1±3.2	38.7±1.5
Spleens 4 and 5	38	15	39.5±5.3	27	8	29.8±5.9	17	4	23.5±6.9	32.9±3.5
All palpable spleens	1,496	606	40.5±0.9	1,043	339	32.5±1.0	441	112	25.4±1.4	35.5±0.6
All exams	2,064	705	34.2±0.7	1,440	408	28.3±0.8	593	149	25.1±1.2	30.8±0.5

There was evidence of a significantly decreasing parasite rate with increasing age in all examinations, all palpable spleen examinations and in examinations of those with spleen sizes 1, 2 and 3, but not in the examinations of those with spleen not palpable nor spleen sizes P and 4 and 5.

From a study of the columns of Table IV it may be stated that there was, in the 0-9 year age group, evidence for an increase of parasite rates with increasing size of spleen up to size 3. This increase was doubtful in the 10-19 year group, and did not exist in the adult group. Any such relationship then in the examinations here reported, may be expected to be evident only in the 0-9 year age group.

In Table V are given the infection rates of the three species of parasites for different sizes of spleens in the 0-9 year age group only. The figures for persons showing infection with more than one parasite were omitted because of their small number.

TABLE V.

*Relations of species infection rates to size of spleen (both sexes, 0-9 year age group).\**

	BENIGN TERTIAN.		MALIGNANT TERTIAN.		QUARTAN.	
	No. inf.	Per cent inf.	No. inf.	Per cent inf.	No. inf.	Per cent inf.
Spleen not palpable ..	44	77±0.7	8	1.4±0.3	46	81±0.8
Spleen P ..	42	14.1±1.4	21	7.1±1.0	35	11.8±1.3
Spleen 1 ..	84	17.6±1.2	25	5.2±0.7	61	12.8±1.0
Spleen 2 ..	109	24.2±1.4	25	5.6±0.7	64	14.2±1.1
Spleen 3 ..	58	24.8±1.9	13	5.6±1.0	35	15.0±1.6
Spleens 4 and 5 ..	11	28.9±5.0	1	2.6±1.7	3	7.9±2.9
All palpable spleens ..	304	20.3±0.7	85	5.7±0.4	198	13.2±0.6
All examinations ..	348	16.9±0.5	93	4.5±0.3	244	11.8±0.5

\* Number of persons examined the same as 0-9 column of Table IV.

The malignant tertian and quartan rates, found in persons showing spleens of sizes 4 and 5 when examined, were not significantly larger than their probable errors. In the case of all three parasites, the rates of infection in persons not having palpable spleens were well below the rates of infection in persons having palpable spleens of any and all sizes, with the exception of the two rates just mentioned. The tendency for infection rates to increase with size of spleen was, however, demonstrable only in the case of benign tertian infections. Therefore, the increase in the general parasite rate of this age group (0-9 years), noted in Table IV, was due mainly to the benign tertian infections found.

The recording of spleen sizes made it possible to judge differences in distributions of these sizes between various classes of persons examined, and for this purpose the chi-square test was used. Since it was impossible, with the method of recording here followed, to arrive at a statistically correct average of spleen size, it became necessary to use some other method of judging differences in distribution. The chi-square test seemed to be eminently suitable for such use, since it would not only determine the significance or otherwise of differences



between two distributions, but would also indicate at what points these differences occurred.

As the spleen rate, with a probable error, gave a method of judging differences in the presence of non-palpable and palpable spleens, only the palpable spleens were included in the chi-square tests. Table VI demonstrates the method used by applying the chi-square test, to judge differences in distribution of spleen sizes between persons who were found to have parasites in their blood at the time of examination and persons not so found.

TABLE VI.

*Chi-square test on distribution of spleen sizes in all persons found to have malaria parasites in the blood at examination and those not so found.*

	Number with spleen P.	Spleen 1.	Spleen 2.	Spleen 3.	Spleens 4 and 5.	TOTALS.
i Infected	179	318	359	174	27	1,057
ii Not infected	421	630	540	277	55	1,923
iii i + ii	600	948	899	451	82	2,980
iv i/1057	0.1693	0.3009	0.3396	0.1646	0.0256	1.0000
v ii/1923	0.2189	0.3276	0.2808	0.1440	0.0287	1.0000
vi iv-v	-0.0496	-0.0267	+0.0588	+0.0206	-0.0031	..
vii (vi) <sup>a</sup>	0.002460	0.000713	0.003457	0.000424	0.000010	..
viii vii/iii	0.0000041	0.0000008	0.0000038	0.0000009	0.0000001	0.0000097

$$\text{Chi-square} = 1057 \times 1923 \times 0.0000097 = 19.72$$

$$\text{Spleen rates :—Infected} = .83.8 \pm 0.7$$

$$\text{Not infected} = 67.8 \pm 0.6$$

The spleen rate for persons found to have parasites in the blood at the time of examination was  $83.8 \pm 0.7$ , while that of those not so found was  $67.8 \pm 0.6$ , a highly significant difference of  $16.0 \pm 0.9$ . The chi-square obtained in testing differences in distribution of palpable spleen sizes was 19.72, which was also highly significant. This demonstrated that the two distributions were not random samples from the same universe but could be regarded as really different. As the plus and minus signs of row (vi) show, this difference was in the direction of more spleens of sizes 2 and 3 and fewer spleens of sizes P and 1 in those found infected. The two classes of persons were approximately equal in their proportions of spleens of the largest sizes, 4 and 5. This was true when individuals under 0-9 years were considered, and it was this age group only which produced this variation. In the 10-19 year age group, although the spleen rates were very different, the distribution of spleen sizes

could not be demonstrated to vary between those found infected and those not found infected. There was also no significant difference between the two classes in either spleen rate or distribution of spleen sizes in the adult age group (*see* Table VII, *a*, *b* and *c*).

Considering all the examinations made, the males had the higher spleen rates and, in distribution of spleen sizes, they had significantly fewer small spleens and more large spleens than the females. There were so few adult females examined that no adult group could be tested, so a class of ten years and over was used. The difference between the sexes in spleen rates and distribution of spleen sizes, was again found to be significant in the 0-9 year group, but not in the older age grouping used (*see* Table VII, *d*, *e* and *f*).

Since there was this difference between the sexes, the remaining spleen rates and chi-square tests of Table VII were calculated on examinations of males only. There was no difference between any of the age groups used in either the spleen rate or the proportionate representation of spleen sizes (Table VII, *g*, *h* and *i*). This was true in both the cases of those in whose blood parasites were found, and of those in whom they were not found, although the figures for these sub-groups were not included in Table VII.

#### RELATIONSHIP BETWEEN SPLEEN ENLARGEMENT AND SPECIES OF PARASITE.

Malaria parasites found in blood slides were recorded by species diagnosed, so it was possible to form sub-groups of infected persons. When all examinations were considered, those found infected with benign tertian parasites had a spleen rate of  $88.1 \pm 1.0$ , while those found infected with malignant tertian had a rate of  $80.5 \pm 2.2$ , a possibly significant difference of  $7.6 \pm 2.4$ . A chi-square of 25.54 showed that the two distributions of spleen sizes were not random samples from the same universe, the difference being in more large and fewer small spleens in the benign tertian group. When two age groups were made of the two parasite groupings, it was found that these differences were not apparent in the 0-9 year group, but were present in the older group of ten years and over. Those found infected with benign tertian parasites had a significantly higher spleen rate than persons found infected with quartan parasites, and there was a shift in spleen sizes to the larger spleens in the benign tertian group. In the 0-9 year group there was a significant difference in spleen rates, the benign tertian infected having the higher rate, but this was not true in the 10 years and over group. A difference in distribution of spleen sizes could be demonstrated in neither of the two age groups, although it was present when all examinations were considered (*see* Table VII, *j*, *k*, *l*, *m*, *n* and *o*).

Between the two groups of persons diagnosed as having malignant tertian and quartan infections, there was no difference in either spleen rates or distribution of spleen sizes (*see* Table VII, *p*). This was true in both the 0-9 year age group and the 10 years and over group.

Diagnoses of infections with more than one species of parasite were few, so no division into age groups could be made. There was no significant difference in spleen rates between those having mixed infections and those stated to have single infections of either benign tertian, malignant tertian, or quartan. Nor did the chi-square tests demonstrate any difference in distribution of spleen sizes, except in the test for mixed infections and malignant tertian infections, where the chi-square was barely significant. In this test the persons found to have malignant tertian infections had higher proportions of the smaller spleens.

### SUMMARY.

In a consideration of parasite rates obtained during a study of malaria in three stations in Mysore State, it was found that, in general, there was a decrease in these rates with advancing age but no difference in rates between sexes except in adults, where males had the higher rates. The decrease with age was shown by the benign tertian infection rates, and to a lesser extent by the quartan rates. It could not be demonstrated in malignant tertian nor mixed infection rates.

Spleen rates for the youngest age group examined (0-4 years) were lower than those for other age groups but no other changes with age could be demonstrated. The rates for females were below those for males, in children under ten and in the total examinations for all ages. The parasite rates in persons not having a palpable spleen at the time of examination, were lower than those in persons with a palpable spleen, only in the 0-9 and 10-19 year age groups. In the adult group of 20 years old and over, there was no difference in parasite rates with presence or absence of palpable spleen, nor with increasing spleen sizes. The 0-9 year age group gave evidence of an increasing parasite rate with increasing size of spleen up to size 3, but this was doubtful in the 10-19 year group. This tendency of the parasite rates was demonstrable only for benign tertian infections. The differences in the palpable spleens of the various groups were further studied by means of the chi-square tests on the proportionate distributions of spleen sizes.

TABLE VII.

*Chi-square tests on the distribution of spleen sizes, and the spleen rates, of various classes of persons examined.*

	Number with Spleen P	Spleen 1	Spleen 2	Spleen 3	Spleens 4 and 5	Total.	Spleen rate.
(a) <i>By infection with malaria parasites—both sexes—0-9 years.</i>							
Infected ..	99	174	207	111	15	606	$86.0 \pm 0.9$
Not infected ..	198	303	243	123	23	890	$65.5 \pm 0.9$
TOTAL ..	297	477	450	234	38	1,496	Chi-square=19.90

TABLE VII—contd.

	Number with Spleen P	Spleen 1	Spleen 2	Spleen 3	Spleens 4 and 5	Total.	Spleen rate.
<i>(b) By infection with malaria parasites—both sexes—10-19 years.</i>							
Infected ..	60	109	117	45	8	339	83.1±1.3
Not infected ..	156	230	205	94	19	704	68.2±1.0
TOTAL ..	216	339	322	139	27	1,043	Chi-square=4.46
<i>(c) By infection with malaria parasites—both sexes—20 years and over.</i>							
Infected ..	20	35	35	18	4	112	75.2±2.4
Not infected ..	67	97	92	60	13	329	74.1±1.4
TOTAL ..	87	132	127	78	17	441	Chi-square=0.92
<i>(d) By sex—all ages—all examinations.</i>							
Males ..	487	791	754	397	75	2,504	74.0±0.5
Females ..	113	157	145	54	7	476	66.9±1.2
TOTAL ..	600	948	899	451	82	2,980	Chi-square=12.75
<i>(e) By sex—0-9 years—all examinations.</i>							
Males ..	204	352	337	187	31	1,111	75.2±0.7
Females ..	93	125	113	47	7	385	65.7±1.3
TOTAL ..	297	477	450	234	38	1,496	Chi-square=9.92
<i>(f) By sex—10 years and over—all examinations.</i>							
Males ..	283	439	417	210	44	1,393	73.0±0.9
Females ..	20	32	32	7	0	91	72.2±2.6
TOTAL ..	303	471	449	217	44	1,484	Chi-square=7.30
<i>(g) By age—males only—all examinations.</i>							
0-9 years ..	204	352	337	187	31	1,111	75.2±0.7
10-19 years ..	199	307	293	134	27	960	72.6±0.8
TOTAL ..	403	659	630	321	58	2,071	Chi-square=4.27
<i>(h) By age—males only—all examinations.</i>							
0-9 years ..	204	352	337	187	31	1,111	75.2±0.7
20 and over ..	84	132	124	76	17	433	74.1±1.2
TOTAL ..	288	484	461	263	48	1,544	Chi-square=2.02

TABLE VII—*contd.*

	Number with Spleen P	Spleen 1	Spleen 2	Spleen 3	Spleens 4 and 5	Total.	Spleen rate.
(i) <i>By age—males only—all examinations.</i>							
10-19 years ..	199	307	293	134	27	960	726±0·8
20 and over ..	84	132	124	76	17	433	741±1·2
TOTAL ..	283	439	417	210	44	1,393	Chi-square=4·49
(j) <i>By parasites—all ages—males only.</i>							
Benign tertian ..	49	123	162	81	15	430	88·1±1·0
Malignant tertian	31	44	26	18	1	120	80·5±2·2
TOTAL ..	80	167	188	99	16	550	Chi-square=25·54
(k) <i>By parasites—0-9 years—males only.</i>							
Benign tertian ..	29	65	90	52	9	245	90·1±1·2
Malignant tertian	10	18	12	10	0	50	90·9±2·6
TOTAL ..	39	83	102	62	9	295	Chi-square=7·22
(l) <i>By parasites—10 years and over—males only.</i>							
Benign tertian ..	20	58	72	29	6	185	85·6±1·6
Malignant tertian	21	26	14	8	1	70	74·5±3·0
TOTAL ..	41	84	86	37	7	255	Chi-square=18·78
(m) <i>By parasites—all ages—males only.</i>							
Benign tertian ..	49	123	162	81	15	430	88·1±1·0
Quartan ..	56	88	93	48	8	293	80·3±1·4
TOTAL ..	105	211	255	129	23	723	Chi-square=9·93
(n) <i>By parasites—0-9 years—males only.</i>							
Benign tertian ..	29	65	90	52	9	245	90·1±1·2
Quartan ..	25	41	44	28	3	141	82·9±1·9
TOTAL ..	54	106	134	80	12	386	Chi-square=4·17
(o) <i>By parasites—10 years and over—males only.</i>							
Benign tertian ..	20	58	72	29	6	185	85·6±1·6
Quartan ..	31	47	49	20	5	152	77·9±2·0
TOTAL ..	51	105	121	49	11	337	Chi-square=7·00

TABLE VII—concl'd.

	Number with Spleen P	Spleen 1	Spleen 2	Spleen 3	Spleens 4 and 5	Total.	Spleen rate.
<i>(p) By parasites—all ages—males only.</i>							
Malignant tertian	31	44	26	18	1	120	$80.5 \pm 2.2$
Quartan ..	56	88	93	48	8	293	$80.3 \pm 1.4$
TOTAL ..	87	132	119	66	9	413	Chi-square=7.51
<i>(q) By parasites—all ages—males only.</i>							
Benign tertian ..	49	123	162	81	15	430	$88.1 \pm 1.0$
Multiple infections	3	6	12	7	0	28	$87.5 \pm 4.0$
TOTAL ..	52	129	174	88	15	458	Chi-square=2.17
<i>(r) By parasites—all ages—males only.</i>							
Malignant tertian	31	44	26	18	1	120	$80.5 \pm 2.2$
Multiple infections	3	6	12	7	0	28	$87.5 \pm 4.0$
TOTAL ..	34	50	38	25	1	148	Chi-square=9.16
<i>(s) By parasites—all ages—males only.</i>							
Quartan ..	56	88	93	48	8	293	$80.3 \pm 1.4$
Multiple infections	3	6	12	7	0	28	$87.5 \pm 4.0$
TOTAL ..	59	94	105	55	8	321	Chi-square=4.47



## NOTES ON MALARIA IN MYSORE STATE.\*

### Part IV.

#### EXPERIMENTAL CONTROL OF MALARIA WITH PARIS GREEN AND PLASMOQUINE.

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### INTRODUCTION.

PARTS I, II and III of this report dealt with the findings of a study of uncontrolled malaria in three stations in Mysore State. The periods covered by these reports were as follows : In the Nagenhalli area, the sixteen months between 1st October, 1928, and 1st February, 1930; in the Mudigere area, the fourteen months from 1st December, 1928, to 1st February, 1930; in the Hiriyur area, the twenty-four months of 1929 and 1930. Subsequent to this period of preliminary observation, experimental control of malaria was begun in the Nagenhalli area on 1st February, 1930, and on 17th February, 1930, in the Mudigere area. In addition to these areas, preliminary observation of malaria and subsequent control by plasmoquine distribution alone were carried on at Marikanave village, twelve miles from Hiriyur town, and studies in the control of *A. stephensi* breeding were made in Bangalore City. Part IV of the notes, and subsequent parts, will report on these experiments in the control of malaria.

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## I. EXPERIMENTAL CONTROL WITH PARIS GREEN AND PLASMOQUINE (NAGENHALLI AREA).

A description of the Nagenhalli area, which will not be repeated here, was given in Part I. Within the area there were four villages and a paddy cultivation station of the Mysore Department of Agriculture. The area was divided into two parts: the first, comprising Nagenhalli village and the farm, was to be the area within which it was decided to attempt control of malaria and was referred to as the 'protected zone'; the second, made up of the remaining three villages, was called the 'peripheral zone'. In August 1930, blood and spleen examinations were begun in Palahally village, two miles from Nagenhalli, to serve as a 'test zone' for the control work.

### CONTROL METHODS.

At the beginning of control experiments it was decided, arbitrarily, to attempt to control breeding of the dangerous species of anophelines within a circle of a one-mile radius from the centre of the protected zone. The dangerous species of anophelines were considered to be *A. culicifacies*, *A. fluviatilis* (*A. listonii*), *A. minimus*, *A. varuna*, and *A. stephensi*, as was mentioned in Part II. Since this circle with the one-mile radius went slightly beyond the village of Lakshmipur and well beyond the villages of Kalastvadi and Siddalingapur, these villages of the peripheral zone were theoretically protected, on at least one side, from invasions of dangerous anophelines. Palahally village, being two miles away, was considered to be entirely unprotected. Malaria control of this one-mile circle was carried on from 1st February, 1930, to 21st March, 1932, when the radius was reduced to one-half mile. A further reduction of the radius to one-quarter mile will be attempted later.

### PARIS GREEN CONTROL.

It was decided to use paris green for the control of anopheline breeding. As the road dust available was somewhat heavy and difficult to spread in a cloud, ash was obtained from a nearby mill and mixed with the road dust in varying proportions, dependent on the strength of the wind during the season of use. The usual proportions were five parts ash to six parts road dust. One per cent of paris green was used in this road-dust-ash diluent and at no time, in this area, was it found necessary to increase the percentage. The road dust and ash were sieved, and mixed with paris green. The equipment employed was copied from that in use in the 'Stazione Sperimentale per la Lotta Antimalarica' in Italy. The mixture was spread by means of the usual hand blower and by direct manual application.

It may be of interest to note here that, at the beginning of control work in all three stations, there were complaints of skin eruptions from the labourers

engaged in mixing and spreading the paris green larvicide. The eruptions occurred principally in the arm pits, inguinal and anal regions. They were at no time serious, and disappeared after the staff had been warned of the necessity of bathing and washing their clothes thoroughly immediately after ceasing work. At no time during the control period have symptoms suggestive of arsenic poisoning appeared in any of the staff, nor have complaints been received of paris green having affected either men or animals in the areas controlled.

At the beginning of control work in the Nagenhalli area, the question of the necessity of spraying paddy fields with the paris green mixture arose immediately. Reference to the description of this area shows that from July to January the greater part of the acreage of the area is under paddy cultivation. The planting begins in June, and by September the paddy is well grown. It was between these months, therefore, during 1928 and 1929, that most of the paddy-field breeding of anophelines occurred. Of all the anopheline larvæ found in paddy fields only about a quarter were of the dangerous species; also, of all the larvæ of the dangerous species caught, calculated as larvæ per one hundred dips, less than one-quarter were caught in paddy fields. Since the heaviest production of paddy-field larvæ was in 'off' months, so far as malaria was concerned; since so small a proportion was of the dangerous species; and since such a large acreage would have required spraying, it was decided not to control paddy-field breeding. This was on the assumption that, even without this control, it would be possible to reduce the dangerous anophelines to a critical level of numbers at which successful maintenance of malaria would become impossible.

This decision reduced the area to be sprayed with paris green to (a) the irrigation channels, large and small, and (b) certain swampy areas created by these channels, by the railway line embankment, and by the main Bangalore-Mysore road. It was estimated that within the control zone there were approximately 25 miles of channels. Some of the larger of these had to be sprayed from both banks, although wherever it was possible spraying was done from the middle.

During the control work the staff of each area consisted of one health officer in charge, two assistant inspectors, one clerk and ten labourers. The number of the latter was reduced when the control zone was reduced in size. The area was divided into four parts (later into three), so that each sub-area was paris-greened once a week, and Saturday was left free for visiting the regular anopheline catching stations. Spleen and blood examinations in the villages of the area were made once a quarter instead of once a month. In order more thoroughly to systematize the work, all spraying was carried out in a routine manner without regard to whether larvæ were found or not. Larval catches on a test basis were made just before and one day after each spraying.

**PLASMOQUINE-QUININE CONTROL.**

As an aid to the larval control in the Nagenhalli area it was decided to use small doses of plasmoquine-quinine compound. For this purpose a grant, obtained from the Mysore District Board, was spent in the purchase of tablets containing  $1/12$  grain plasmoquine and one grain quinine. The tablets were distributed once a week in the protected zone, children between two and ten years receiving one tablet and persons over ten receiving two. Since the population concerned was a civil one which allowed of no compulsion, the taking of the tablets was entirely voluntary and it was found very difficult to get the co-operation of the villagers. In no week did more than forty per cent of the total population take the tablets and in some weeks only eight per cent could be persuaded. The average weekly percentage of the people taking the dose was but twenty-six. The distribution of tablets began on 3rd March, 1930, and was stopped on 1st July, 1932, because the grant was not renewed and because so small a proportion of the people took the dose. It should be mentioned that at no time were tablets left for absentees or for consumption at some other time. All tablets accepted were swallowed in the distributor's presence.

Both the methods of control, paris green spraying and plasmoquine distribution, were used twelve months a year. Up to the present time no attempt has been made to reduce the number of months of control in the Nagenhalli area.

**RAINFALL AND TEMPERATURE RECORDS.**

Tables I and IV of Part I contain the rainfall and temperature records for Nagenhalli area for the years 1929, 1930 and 1931. Therefore only the record for 1932 and the yearly totals and averages for the previous years will be given here (Table I). The figures for the average annual rainfall were taken from the records of Mysore City, four miles from Nagenhalli.

During the year 1932, the annual rainfall was practically equal to the average, the maximal falls coming in May, August and October. The yearly range of temperature was slightly greater than during the previous three years and the average 8 a.m. relative humidity was higher. There had been a deficient rainfall during the year of observation, an excess during the first year of control, and a deficiency during the second. There seemed, therefore, to be no decided change of rainfall, temperature or humidity factors which might have led to a decrease in malaria.

**SPLEEN AND PARASITE RATES.**

During the period of observation examinations to establish spleen and parasite rates were made once a month, but after the beginning of control work this interval was increased to three months. As submission to examination was on a purely voluntary basis, and as it became increasingly difficult to get

TABLE I.

*Rainfall and temperature records of Nagenhalli for 1932 and summaries of previous years (see Tables I and IV of Part I).*

Months.	Average rainfall 35 years (inches).	Rainfall (inches).	Maximum in 24 hours (inches).	Number of days of rain.	Maximum temperature registered (°F.).	Minimum temperature registered (°F.).	Mean temperature (°F.).	Average 8 a.m. relative humidity (per cent).
January ..	0'15	0'00	0'00	0	88'0	57'0	71'4	81'2
February ..	0'16	0'00	0'00	0	93'0	57'0	77'5	82'1
March .	0'48	0'00	0'00	0	98'0	60'0	81'4	76'7
April ..	2'44	1'74	1'38	3	98'0	67'5	82'0	73'0
May .	5'09	7'13	2'60	10	98'0	67'0	78'8	77'5
June .	2'74	0'68	0'35	4	89'0	62'0	74'3	79'8
July ..	2'69	1'72	0'43	12	88'0	66'0	73'0	83'8
August ..	3'11	6'10	2'15	10	93'0	66'5	77'3	79'1
September ..	4'72	0'57	0'23	4	90'0	64'0	74'9	85'8
October ..	5'93	7'89	2'58	16	88'0	65'0	76'8	87'1
November ..	2'58	4'35	1'10	9	87'0	62'0	74'5	87'1
December ..	0'34	0'00	0'00	0	87'5	54'0	72'8	85'0
For year .	30'43	30'18	2'60	68	98'0	54'0	76'2	81'5
1929 ..	..	24'52	2'17	57	100'0	59'0	76'9	76'4
1930 ..	..	31'75	1'55	55	100'0	58'0	77'4	74'0
1931 ..	..	23'78	2'90	79	98'0	58'0	76'6	76'3

volunteers, it was necessary to use examinations of all ages for obtaining rates, in order that these might be as significant, statistically, as possible. For this part of the notes, therefore, and for subsequent parts, the spleen and parasite rates represent all ages examined, unless there is specific mention otherwise.

It was mentioned in Part III that there was a tendency for parasite rates to decrease with increasing age in the examinations of all three areas. This tendency was not apparent in the case of spleen rates. From this one might expect the all-ages parasite rates to vary with the proportion of children in the

0-9 year group represented in each series examined. In the protected zone of the Nagenhalli area the proportion of such children in the yearly examinations increased year by year from 34 per cent during the observation period to 45 per cent in 1932. In the peripheral zone the change was from 49 to 75 per cent, and in Palahally village from 74 to 86 per cent. The expected result of these increases in the proportion should be in the direction of increasing the parasite rates in all three zones.

Graph 1 gives the all-ages spleen and parasite rates of the three zones in the Nagenhalli area from the beginning of observation in October 1928 up to date (May 1933). The spleen rates of the protected zone varied between 70 and 90 per cent from October 1928 to April 1930; between 40 and 50 per cent from July 1930 to August 1931, and between 27 and 41 per cent since that time. Paris green control work began on 1st February, 1930, and plasmoquine distribution on 3rd March, 1930, the latter being stopped on 1st July, 1932. The first break in the spleen rates came three months after the beginning of paris green work and two months after the beginning of plasmoquine distribution; the second break, 17 and 16 months respectively after the beginnings of the two control measures. It cannot as yet be said that the cessation of plasmoquine control had any effect on the spleen rates.

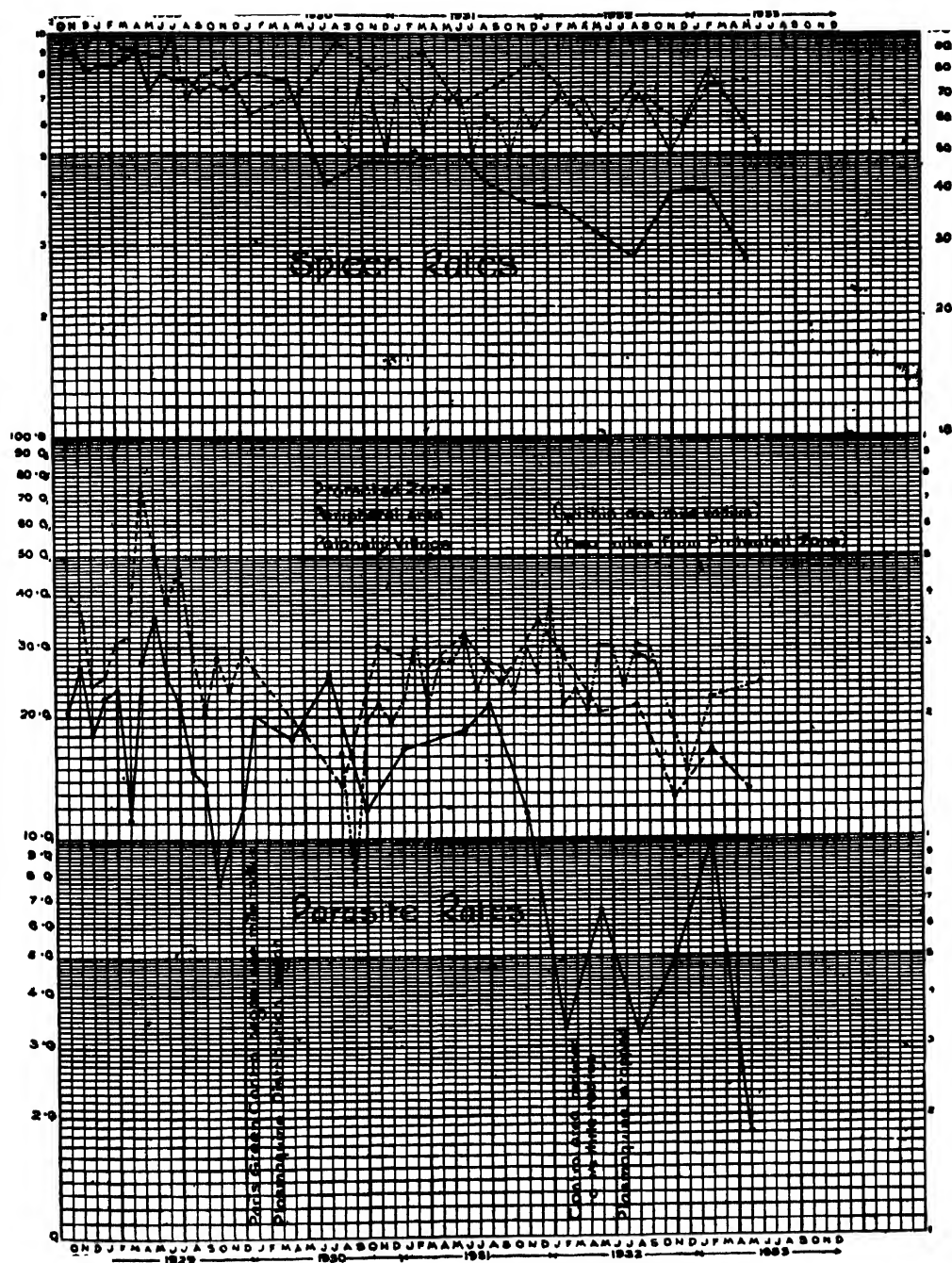
The figures for the peripheral zone, which it will be remembered was considered to be partially protected by the paris green work but which received no plasmoquine, show variations for the same period of 63 to 100, 68 to 96, and 51 to 86 per cent respectively. There would seem to have been some slight tendency downwards, but not nearly so great a tendency as that exhibited by the protected zone spleen rates. The reduction of the paris green control area on 16th February, 1932, from a one-mile to a half-mile radius, appears to have had no effect on the spleen rates of either the protected or peripheral zone.

Each successive maximum parasite rate in the protected zone was somewhat lower than the preceding one, the maxima being in May 1929, July 1930, and August 1931. Subsequent to the last-named date, nineteen months after the beginning of paris green control and eighteen months after that of plasmoquine distribution, there was a sharp drop in the parasite rate, which did not rise above 10 per cent after the November 1931 examination. In the peripheral zone also there was a sharp drop in the parasite rates from a maximum in April 1929 to a minimum in August 1930, after which they rose to continue between 20 and 40 per cent until the examinations of August 1932. From that date the rates vary between 10 and 20 per cent. The reduction of the paris green control area cannot be said to have had any effect on either of these rates, nor the cessation of plasmoquine distribution on the parasite rates of the protected zone.

Examinations were begun in Palahally village in August 1930, and were made every month for twenty-six months, after which they were made quarterly. The spleen rates for this village, which was considered to be entirely unprotected and is situated about two miles from Nagenhalli village,

# NAGENHALLI AREA.

GRAPH 1.



remained between 50 and 80 per cent throughout, and seemed to have no tendency to decline. With the exception of examinations made in August, September, October and December 1930, and in December 1932, all resulting parasite rates for this village were between 20 and 40 per cent, and showed no tendency to decrease.

It would seem possible to conclude from the findings to date that (a) there was no natural reduction in malaria in this area; (b) that the partial protection afforded the villages of the peripheral zone by the paris green work resulted in a very slight reduction in spleen rates and a somewhat more marked reduction in parasite rates; and (c) that the paris green and plasmoquine control of malaria in the protected zone resulted in a marked reduction in both spleen rates and parasite rates. This reduction took place in an irrigated rural area, in which so far as can be ascertained there was no change either in living conditions, diet, or irrigation habits, and in which no engineering control was attempted.

#### REDUCTIONS IN PARASITE RATES.

Examinations of spleens and bloods in the protected zone were made in January, April, July and October 1930; in January, June, August and November 1931; and in February, May, August and November 1932. In order to compare

TABLE II.

*Results of yearly blood examinations in the protected zone subsequent to control work and for corresponding months of 1928-29. Rates for all ages.*

Years.	Number of persons examined	BENIGN Tertian.	MALIGNANT Tertian.		ALL INFECTIONS *	
		Per cent infected.	Per cent infected.	Per cent infected	Number infected	Per cent infected
1928-29a †	293	12.3±1.3	3.4±0.7	4.8±0.8	60	20.5±1.6
1928-29b †	261	10.0±1.2	2.7±0.7	8.0±1.1	54	20.7±1.7
1928-29c †	281	11.4±1.3	3.6±0.7	8.2±1.1	66	23.5±1.7
1930	125	8.0±1.6	1.6±0.8	7.2±1.6	21	16.8±2.3
1931	141	6.4±1.4	0.7±0.5	8.5±1.5	22	15.6±2.0
1932	221	0.4±0.3	0.4±0.3	3.6±0.8	10	4.5±0.9

\* Including mixed infections, not given elsewhere in Table.

† 1928-29a = January, April, July and October to be compared with examinations in the same months in 1930. 1928-29b = January, June, August and November to be compared with examinations made in 1931. 1928-29c = February, May, August and November to be compared with examinations made in 1932.

the resulting parasite rates with those found during 1928-29, it was necessary to use the figures for the same months in both years to be compared. Table II gives the results of all examinations made in 1930, 1931 and 1932, and those for the three corresponding groups of months of 1928-29.

There was no significant difference between the all-infection rates of 1928-29a and of 1930, nor between these rates for 1928-29b and 1931. The difference between the  $23.5 \pm 1.7$  rate of 1928-29c and the  $4.5 \pm 0.9$  rate of 1932 was  $19.0 \pm 1.9$  which was a highly significant reduction. The differences in the rates between 1930 and 1932, and 1931 and 1932, were also significant but the 1930 and 1931 rates did not differ from each other.

The benign tertian parasite rate for 1932 and the malignant tertian rates for 1931 and 1932 were not significantly greater than their probable errors, but since they were in line with a general decline, which sooner or later would be sure to produce more such non-significant rates, it was considered that they could be used in determining reductions. There was no significant difference between any of the species parasite rates of 1928-29a and 1930, nor between those of 1928-29b and 1931, but all of the species rates of 1932 were significantly lower than those of 1928-29c. For the control years, although the months in which examinations were made differed somewhat, there was no significant change between 1930 and 1931, but there was a decided drop in benign tertian rates from 1930 to 1932 and from 1931 to 1932.

Although the relative table will not be reproduced here, it may be stated that in the villages of the peripheral zone there was a significant moderate drop in total parasite rates between 1928-29a and 1930, and between 1928-29c and 1932, but not in the case of 1928-29b and 1931. There were no significant changes in species parasite rates for the years just mentioned, except in the case of the benign tertian rates of 1928-29c and 1932 where there was a moderate drop. There were also no significant changes between any of the rates of 1930, 1931 and 1932.

In the Palahally village examinations only the yearly results of 1931 and 1932 are fairly comparable. There was no significant change in these two years' rates in the 'all infection' parasite rates, nor in those for quartan or malignant tertian, but there was a small drop in the benign tertian rate.

Considering only the years 1931 and 1932, for which rates were available for all three zones of the area, it may be stated that there was no natural reduction of all-species rates evident, but there did appear to be a small natural drop in benign tertian rates. This natural drop was more pronounced in the protected zone, which also was the only zone having a significant drop in the all-species parasite rates.

#### REDUCTIONS IN SPLEEN RATES AND SIZES.

Using the same groups of months as for Table II, the spleen rates and sizes for 1928-29 and for 1930, 1931 and 1932 are given in Table III. There was a significant difference in spleen rates of  $22.5 \pm 3.4$  between 1928-29a and



1930; of  $38.6 \pm 3.3$  between 1928-29b and 1931; and of  $45.1 \pm 2.9$  between 1928-29c and 1932, the difference being in each case a drop in rates to the later year. There was also a significant drop of  $19.6 \pm 4.1$  between the rates for 1930 and 1931, and of  $28.8 \pm 3.7$  between 1930 and 1932, but no significant difference between the 1931 and 1932 rates.

For the examinations in the peripheral zone (no table given), there was no significant difference between the spleen rates for 1928-29a and 1930, but there was a reduction of  $12.1 \pm 3.0$  between 1928-29b and 1931, and of  $28.6 \pm 3.0$  between 1928-29c and 1932. In this zone there was no significant difference between the rates for 1930 and 1931, but there were reductions between 1930 and 1932, and 1931 and 1932, of  $16.7 \pm 3.8$  and  $15.4 \pm 3.8$  respectively. There were no significant changes in the rates determined on the total annual examinations in Palahally village.

TABLE III.

*Numbers in spleen size classes and spleen rates for examinations made in the protected zone during control years and corresponding months of 1928-29. Examinations of persons of all ages.*

Year.	Number of persons examined.	NUMBER IN SPLEEN SIZE CLASSES.						Spleen rates.
		P.	1.	2.	3.	4 and 5.	Total.	
1928-29a *	261	41	35	80	56	11	223	$85.4 \pm 1.5$
1928-29b *	210	26	40	57	41	8	172	$81.9 \pm 1.8$
1928-29c *	236	18	51	65	46	7	187	$79.2 \pm 1.8$
1930	116	9	21	23	20	0†	73	$62.9 \pm 3.0$
1931	141	12	31	11	7	0†	61	$43.3 \pm 2.8$
1932	217	27	27	14	6	0†	74	$34.1 \pm 2.1$

\* See foot-note on page 696 under Table II.

† For the chi-square tests one was taken off the previous spleen size and placed in this cell instead of zero.

The methods of classifying spleen sizes and of using the chi-square test to determine differences in distribution were described in Part III of these notes. The chi-squares of the various comparable rows were as follow :—

1928-29a and 1930—chi-square equals 8.20.

1928-29b and 1931—chi-square equals 20.80.

1928-29c and 1932—chi-square equals 37.85.

The highly significant last two chi-squares resulted from a decided reduction in the numbers of spleens in all classes above size one. This difference was

not evident in the 1930 examinations, although there had been a significant reduction in the spleen rate.

Using the same three periods, it was not until 1928-29c and 1932 were compared that a chi-square test of the peripheral zone examinations could be regarded as significant (23.49). The change in distribution was in the same direction as that of the protected zone. The months in which examinations were made in Palahally village were comparable during 1931 and 1932 only, and there was no significant difference in distribution of spleen sizes between these two years as determined by the chi-square test.

### ANOPHELINE CATCHES.

As was stated in Part II of these notes, regular stations were selected in both the protected and peripheral zones for the catches of adult anophelines on a time basis, twenty minutes being the period spent in each station. From 1st October, 1928, to 1st February, 1930, catches were made twice a week and thereafter only once a week. Therefore the figures for the earlier period were corrected to make them comparable to the later figures. Graph 2 gives the average catch per station per month of females of the so-called dangerous anopheline species. A study of the average catches of the first period of observation shows that there are two peaks, one during the first six months of the year and the other during the last six. The successive peaks in the protected zone for the first six months of the years from 1929 to 1933 inclusive are 12, 17, 3.1, 2.6, and 2.5 respectively by years; the same figures for the peripheral zone are 22, 16, 7, 7, and 13. Corresponding peak average catches for the last six months of the year in the protected zone are 27, 17, 12, and 8; and in the peripheral zone, 34, 27, 36, and 27. (Figures are not as yet available for the last six months of 1933 at the time of writing.) The effect of the larval control in the protected zone was to suppress the peak of the first six months, and year by year to reduce materially the peak of the second six months. In the peripheral zone the peaks of the first six months show the effect of the reduction in control area made in February 1932; the second six months peaks remain practically the same throughout.

For a further study of reductions in catches Table IV was prepared to give the average catches per catching station per month (for the three groups of months used in Part I). The average catch of females of the dangerous species and of all other anopheline species in both the protected and peripheral zones are shown separately.

In the protected zone, in the months of group I, there was a reduction in the average catches of dangerous anophelines of 81 per cent with a maximum reduction in any year of 89 per cent from the 1929 averages. Whereas in the peripheral zone the reduction for those months was 50 with a corresponding maximum of 67 per cent also in 1931. In the months of group II the maximum reduction in the protected zone was 67 as against 14 per cent in the peripheral zone. The corresponding figures for the months of group III were 64 and 5

# NAGENHALLI AREA.

GRAPH 2.

Average catch of dangerous anophelines per station per month.

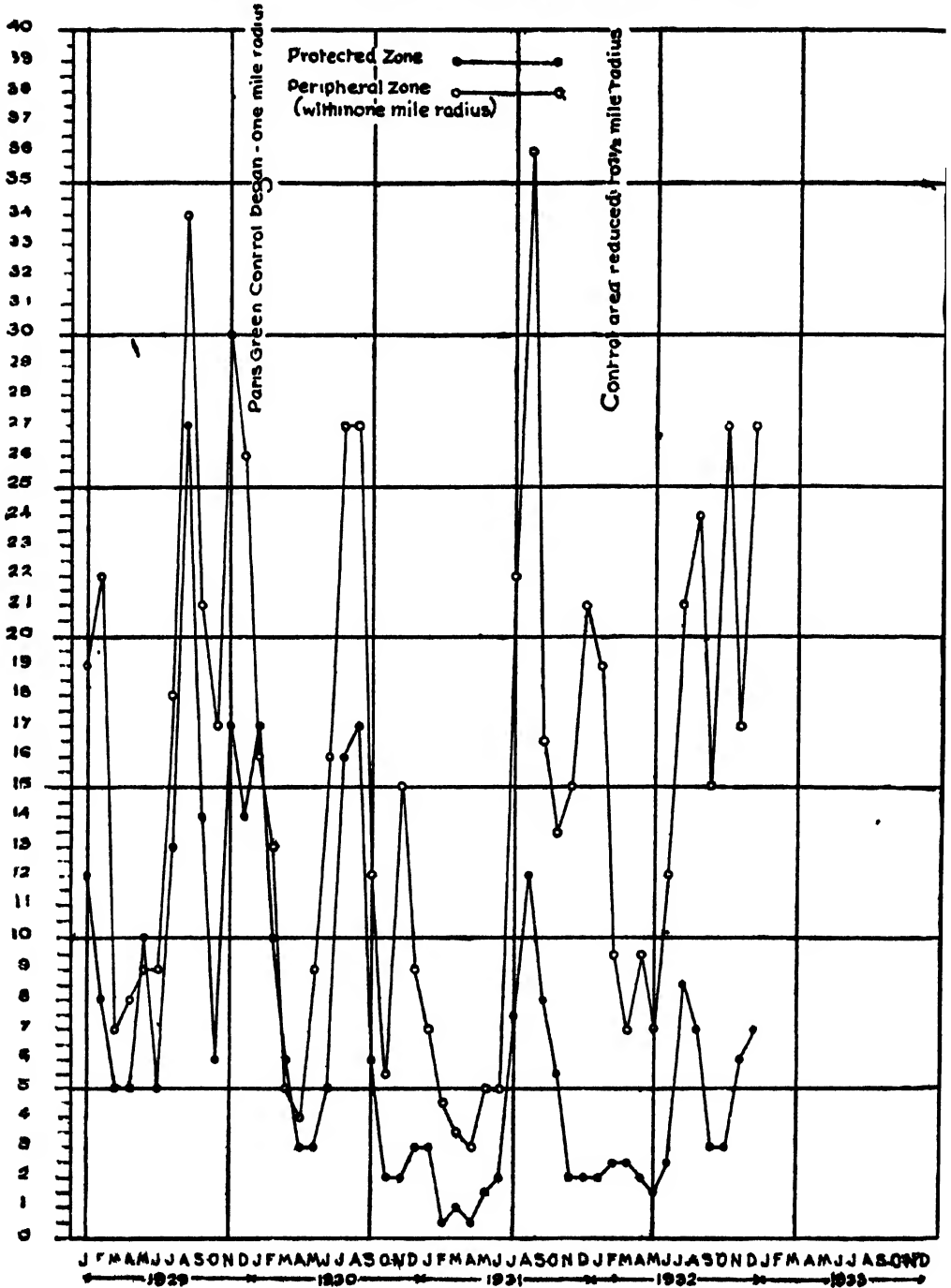


TABLE IV.

Average catch of female anophelines per catching station per month and percentage reduction from first to last year available.

		* Group of months	1929-30.	1930-31.	1931-32.	1932-33.	1933.	Percentage reduction from 1929.
Protected zone	Dangerous anopheline species.	I	7	5	08	21	13	81
		II	15	11	7	5	..	67
		III	14	3	3	5	..	64
	All other anopheline species.	I	8	7	5	4	8	none
		II	10	10	14	12	..	25
		III	13	7	16	14	..	none
Peripheral zone	Dangerous anopheline species.	I	12	8	4	8	6	50
		II	21	21	20	18	..	14
		III	22	9	17	21	..	5
	All other anopheline species.	I	18	14	10	15	18	none
		II	24	19	19	23	..	4
		III	13	11	16	20	..	none

\* I = February, March, April and May.

II = June, July, August and September.

III = October, November, December and January.

per cent, with maximal reductions in any year of 79 and 59 per cent respectively. The control of anophelines was at all times much more effective in the protected zone than in the peripheral and was most effective in the months of group I, which are possibly the most important. There was no evidence in the protected zone of any increase in average catches per month, due to the reduction of the control area early in 1932, in the months of groups I or II. The slight increase for the months of group III may have been due to this fact, although it does not seem probable if there was no effect in the other months.

As compared with the dangerous species there was little or no reduction in average catches of females of other anopheline species in either the protected or the peripheral zone. The maximum reduction from the 1929 averages was 50 per cent in the months of group I of 1932 in the protected zone.

The average catch per station per month for the twelve months of 1929 of females of *A. culicifacies* was 13, while in 1932 it was at its lowest point of

2.3, a reduction of 82 per cent. For females of the *fluviatilis* (*listonii*) group the corresponding figures were 8 and 1.6, a reduction of 80 per cent, but the lowest average was 0.6 in 1931, a reduction of 94 per cent. It was the general experience in all areas that it was easier to control the breeding of the *fluviatilis* (*listonii*) group than that of *culicifacies*.

On the assumption made earlier that about twenty-five per cent of the dangerous anophelines were breeding in the paddy fields then existing in the second half of the year, it might have been expected that at best only about a 75 per cent reduction would be possible in these months, since paddy fields were not controlled. The greatest change from the 1929 figures for those months was in 1930-1931 when there was a 79 per cent reduction, although the 1932 figures for months of groups I and II together showed a reduction from 1929 of only 65 per cent. It was not found possible to keep track of the acreage under paddy cultivation in the control area from year to year.

## II. EXPERIMENTAL CONTROL WITH PARIS GREEN ONLY (MUDIGERE AREA).

A description of the Mudigere area was given in Part I of these notes. Included in the area were the town of Mudigere and the villages of Old Mudigere and Hesgal, the town being the protected zone and the two villages being the partially protected peripheral zone. Old Mudigere is a somewhat scattered village lying across the valley from the town at distances varying from one-half to one-quarter mile; the village of Hesgal, more compact, is about a mile from Mudigere town and across the valley to the north.

### CONTROL METHODS.

Larval control only was used in the Mudigere area. The period of observation in this area extended from 1st December, 1928, to 17th February, 1930, when larval control with paris green began in a circle with a one-mile radius from Mudigere town. The radius was reduced to one-half mile on 21st March, 1932. From the beginning of the control work paris green spraying was carried on continuously until 9th November, 1931, when it was stopped, beginning again on 4th January, 1932. Since then larval control has been on a six months basis with the period between 1st July and 1st January not controlled. At no time was any other than the larval method of malaria control used, but the dispensary continued to be available to all applicants as had been the case for years.

Methods of mixing and spraying paris green were the same in this area as in the Nagenhalli area just discussed, and breeding in paddy fields was not controlled. At times it was necessary to increase the percentage of paris green in the road-dust-ash mixture used in this area, the highest percentage being three per cent for the control of *fluviatilis* (*listonii*) breeding in the river when the flow of water was rapid and heavy.

## RAINFALL AND TEMPERATURE.

The rainfall, temperature and humidity records of this area for 1929, 1930 and 1931 were given in Tables II and V of Part I. Table V gives the records for 1932 and a summary of previous years.

TABLE V.

*Rainfall and temperature records of Mudigere town for 1932 and summaries of previous years (see Tables II and V of Part I).*

Months.	Average rainfall years (inches).	Rainfall (inches).	Maximum in 24 hours (inches).	Number of days of rain.	Maximum temperature registered (°F.).	Minimum temperature registered (°F.).	Mean temperature (°F.).	Average 8 a.m. relative humidity (per cent).
January ..	0'14	0'00	0'00	0	92'0	56'0	73'3	74'0
February ..	0'10	0'00	0'00	0	94'0	57'0	74'9	82'9
March ..	0'45	0'00	0'00	0	98'0	63'0	80'3	69'0
April ..	2'44	4'65	2'31	8	94'5	67'0	79'2	84'8
May ..	4'68	10'51	2'04	18	92'0	65'0	75'9	89'9
June ..	19'16	8'46	0'98	20	84'0	64'0	71'1	91'8
July ..	30'77	44'30	5'67	30	80'0	65'0	69'5	95'4
August ..	16'58	13'29	2'10	20	86'5	65'5	73'5	93'0
September ..	9'14	13'69	1'98	25	84'0	63'5	70'4	93'9
October ..	8'40	13'31	1'54	21	86'5	64'5	74'4	92'7
November ..	3'11	3'37	0'77	12	87'0	59'5	73'6	87'1
December ..	0'68	0'00	0'00	0	91'0	56'5	73'5	83'6
For year ..	95'65	111'58	5'67	154	98'0	56'0	74'1	86'5
1929 * ..	..	92'05	8'30	145	92'0	59'0	72'7	86'4
1930 ..	..	72'18	5'90	123	98'0	55'0	74'9	85'5
1931 ..	..	110'26	5'93	147	97'5	55'0	74'8	86'7

\* Last 8 months of year only for temperatures and 11 months for humidities

The rainfall for 1932 was about sixteen inches in excess of the average, the greatest changes being excesses in April, May, July, September and October

and deficiencies in June and August. Temperatures and humidities were practically the same as in previous years. During the year of observation and the first year of control work rainfall was deficient, especially so in 1930, but since then the annual rainfall has been in excess of the average. In both 1929 and 1931 there were excess rainfalls in April of 7·22 and 5·69 inches respectively; rainfall was about normal in May and June 1929 but very deficient in 1931 in these months, and in 1930 it was deficient in April and normal in May and June.

### DEATH AND DISPENSARY RECORDS.

As was mentioned in Part I, deaths are reported under eight general causes, that of 'fevers' being possibly the best one to use in order to approximate the deaths due to malaria. It was also mentioned that as Mudigere had a dispensary, figures as to clinical diagnoses of malaria in attending patients were available. Table VI gives for the past eight years the percentage of 'fever' deaths to all deaths, and the percentage of malaria diagnoses in all patients attending the dispensary.

TABLE VI.

*Percentage of 'fever' deaths to all deaths and percentage of diagnoses of malaria in dispensary patients for eight years in Mudigere town.*

Years.	Number of deaths all causes.	Number of 'fever' deaths.	Per cent 'fever' deaths.	Total number of dispensary patients.	Number diagnosed as malaria.	Per cent malaria diagnoses
1925 ..	30	23	76·7±5·2	8,037	3,389	42·2±0·4
1926 ..	72	49	68·1±3·7	9,805	5,907	60·2±0·3
1927 ..	57	43	75·4±3·8	9,408	5,196	55·2±0·3
1928 ..	81	75	92·6±2·0	8,059	4,091	50·8±0·4
1929 ..	36	30	83·3±4·2	7,039	2,667	37·9±0·4
1925-29 ..	276	220	79·7±1·6	42,348	21,250	50·2±0·2
1930 ..	44	14	31·8±4·7	8,367	2,246	26·8±0·3
1931 ..	48	7	14·6±3·4	10,221	3,926	38·4±0·3
1932 ..	55	5	9·1±2·6	7,490	2,112	28·2±0·3
1930-32 ..	147	26	17·7±2·1	26,078	8,284	31·8±0·2

There was a very marked reduction in the percentage of 'fever' deaths in the total deaths reported, from 79·7 per cent for the five years previous to 1930

to 17.7 per cent for the three years following, and a highly significant reduction of  $18.4 \pm 0.3$  in the percentage diagnoses of malaria for the same two periods. All the deaths reported were of residents of Mudigere town, but the dispensary patients were from surrounding villages as well as from the town. Just what proportion of these patients were from the town itself was impossible to determine for the earlier years, but the percentage of malaria diagnoses in town patients only was  $29.3 \pm 0.3$  in 1931 and  $18.4 \pm 0.3$  in 1932. It seemed highly probable that the presence in this area of the malaria unit and the necessary publicity attached thereto resulted in more care being taken both in the registering of the cause of death and in the diagnosis of malaria, but how much difference this effected was impossible to estimate. However, it did not seem likely that all of the reduction was due to this influence. It was concluded, therefore, that these reports gave evidence of a decrease of malaria in the town at least, and possibly in the surrounding villages.

### SPLEEN AND PARASITE RATES.

Graph 3 gives the spleen and parasite rates of the Mudigere area examinations made since December 1928, rates being for all ages of persons examined. The spleen rates established by examinations in Mudigere town were found to fall into four groups: Between December 1928 and September 1929 the rates remained between 62 and 91 per cent; between October 1929 and May 1931 the rates varied between 43 and 70 per cent with a tendency to a gradual decline; from June 1931, to July 1932 they rose again to vary between 60 and 76 per cent with the peak in July 1931, and tended to decline thereafter; subsequent to the July 1932 examination there was a decline in rates for each succeeding examination. This was not true in any way for the spleen rates of the peripheral zone which remained fairly constant between 72 and 98 per cent, but by a smaller rise they gave the same evidence of an increase of malaria in the middle months of 1931 as did the rates of the protected zone.

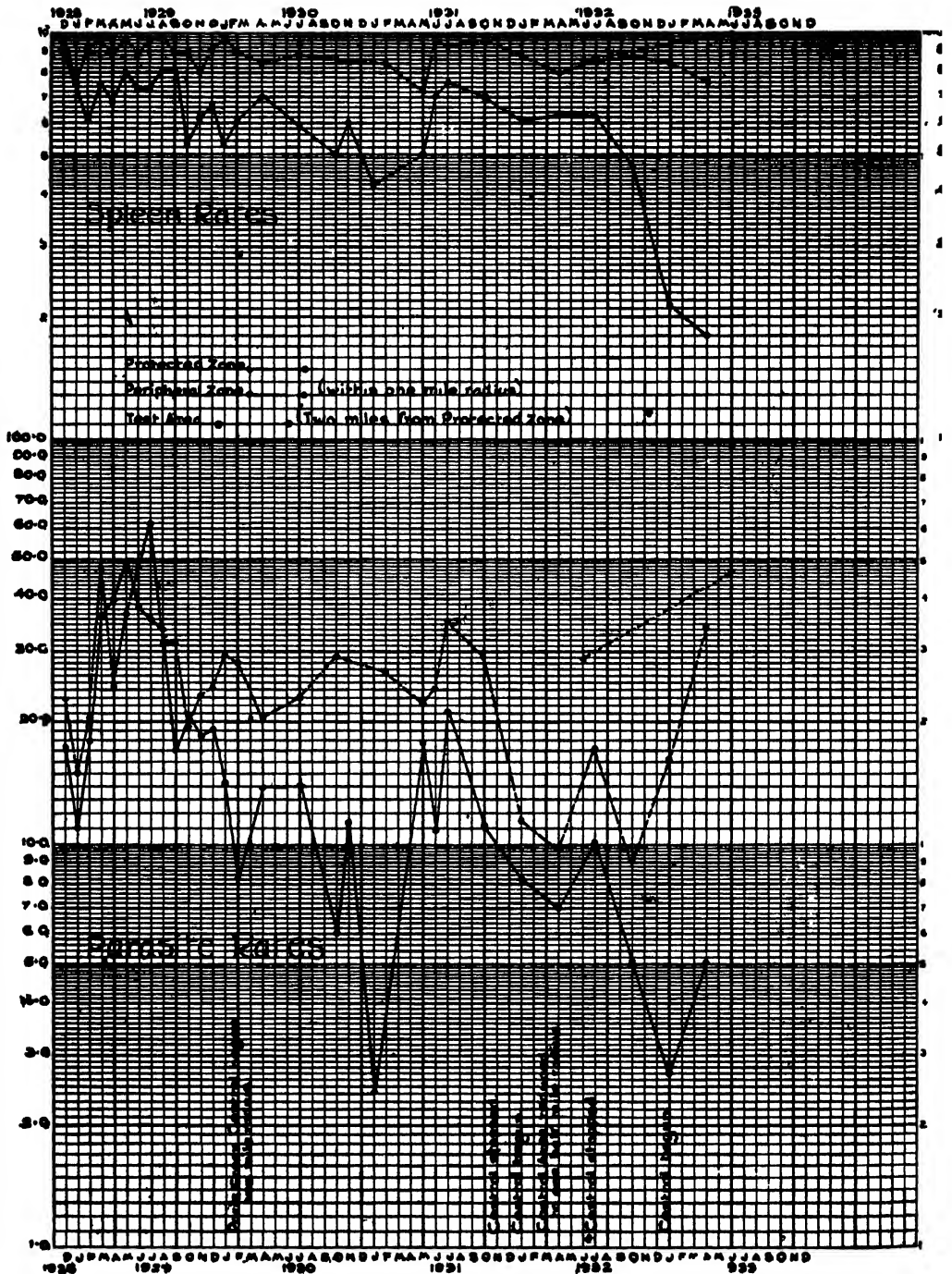
Two villages about two miles from the protected zone were selected as a test area and examinations were made in June 1932 and again in June 1933. The spleen rates established by these examinations agreed with those of the peripheral zone.

The parasite rates of the protected zone showed the same tendencies as the spleen rates, with a drop in rates before control work began (shared by the peripheral zone rates), and a further drop to a low point in January 1931 (not shared by the peripheral zone rates). Subsequent to January 1931 there was a marked increase in parasite rates in the protected zone and a moderate increase in the peripheral zone, followed by a decrease in both rates. The peripheral zone rates, however, showed a marked increase from a low point for October 1932, which was not shared by the rates for the protected zone. The test area rates seemed to confirm the rise shown by those of the peripheral zone.



# MUDIGERE AREA.

## GRAPH 3.



From the above there would seem to have been some natural decline in the malaria of the Mudigere area before the beginning of control work, but this decline was continued in the protected zone only due to larval control. Subsequent to January 1931 there must have been some loss of control resulting in a recurrence of the malaria. This rise was evident throughout the area and was due mainly to an increase in infections with the malignant tertian parasite. Whereas in May, June, July and October 1929 this parasite was found in the proportion of but 9.4 per cent of all parasites, and of 18.2 per cent in 1930, the percentage for these four months of 1931 was 55.3 per cent. Effective control of breeding was apparently resumed, since the rise in rates in the peripheral zone during the latter part of 1932 and early 1933 was not duplicated in the protected zone. There was no apparent effect either from stopping the control work during the last two months of 1931 and the last six months of 1932, or from reducing the area controlled.

## REDUCTIONS IN PARASITE RATES.

Examinations to determine spleen and parasite rates were made in February, April, July, October and November 1930; January, May, June, July and October 1931; January, April, July and October 1932. Table VII gives the results of all examinations made in these years and in corresponding months of 1929 for the protected zone only. There were significant differences between

TABLE VII.

*Results of yearly blood examinations in the protected zone subsequent to control and for corresponding months of 1929. Rates for all ages.*

Years.		Number of persons examined.	BENIGN TERTIAN.	MALIGNANT TERTIAN.	QUARTAN.	ALL INFECTIONS.*	
			Infected per cent.	Infected per cent	Infected per cent.	Number infected.	Per cent.
1929a †	..	358	19.0±1.4	0.8±0.3	2.0±0.5	81	22.6±1.5
1929b †	..	310	23.9±1.6	2.6±0.5	4.5±0.7	97	31.3±1.7
1929c †	..	208	18.3±1.8	1.0±0.5	2.9±0.8	49	23.6±2.0
1930	..	286	3.8±0.8	1.7±0.5	3.8±0.8	29	10.1±1.2
1931	..	379	2.9±0.6	6.9±0.8	2.1±0.5	47	12.4±1.1
1932	..	319	2.2±0.5	3.4±0.7	1.9±0.5	25	7.8±1.0

\* Includes mixed infections, not given elsewhere in Table.

† 1929a = Examinations made in February, April, July, October and November to be compared with examinations of 1930. 1929b = January, May, June, July and October to be compared with 1931 examinations. 1929c = January, April, July and October to be compared with 1932 examinations.

the all-parasite infection rates of 1929 and those for subsequent years, the reductions being  $12.5 \pm 1.9$  for 1930,  $18.9 \pm 2.0$  for 1931 and  $15.8 \pm 2.2$  for 1932. There were no significant differences between these rates for 1930 and 1931 or between 1930 and 1932, but the reduction of  $4.6 \pm 1.5$  between the 1931 and 1932 all-parasite rates may be considered significant in view of the further reduction in the early months of 1933.

In the species parasite rates there was a large significant reduction between the benign tertian rates of 1929*a* and 1930, 1929*b* and 1931, and 1929*c* and 1932, but no significant change after the initial drop of 1930. There was no change in the malignant tertian rate of 1929*a* and that of 1930, nor any significant change between these rates for 1929*c* and 1932; the increase of  $4.3 \pm 0.9$  from the 1929*b* rate to that of 1931 was significant, however. For the years 1930 and after, there was a significant increase in the 1931 rate over that of 1930, and a probably significant decrease from the high point of 1931 to the 1932 rate of  $3.4 \pm 0.7$ . There were no significant changes in the comparable quartan rates.

A comparison of similar rates for the peripheral zone showed decreases in the benign tertian rates, which were of much the same magnitude as in the protected zone with a further significant drop from  $9.5 \pm 1.4$  in 1931 to  $3.2 \pm 1.1$  in 1932. The malignant tertian rates in the peripheral zone showed the same significant rise from the 1929*b* to the 1931 rate as did the protected zone, but did not show a significant drop from the  $10.7 \pm 1.5$  rate of 1931 to the  $6.4 \pm 1.5$  rate of 1932. Quartan rates for the peripheral zone rose from  $3.1 \pm 0.8$  in 1929*a* to  $12.2 \pm 1.8$  in 1930 (a significant rise not found in the protected zone), but showed no significant differences between the rates of 1929*b* and 1931, nor between those of 1929*c* and 1932. The all-species parasite rates in the peripheral zone were not significantly different for 1929*a* and 1930, nor for 1929*b* and 1931, but showed a significant reduction of  $13.2 \pm 2.7$  between the rates of  $25.2 \pm 1.8$  for 1929*c* and  $12.0 \pm 2.0$  for 1932.

There would seem to have been a natural decrease in benign tertian infections in this area, which decrease was significantly more marked in the protected zone than in the peripheral zone in the 1931 examinations, and again in the first two examinations of 1933. There was an increase in malignant tertian infections in 1931 with a subsequent decrease which was more marked in the protected zone, but in no year were the two zones significantly different in these rates. Quartan infections increased in 1930 and decreased in 1932 in the peripheral zone, but showed no changes in the protected zone. The all-species parasite rates of the protected zone were significantly lower than those of the peripheral zone in October and November 1930, January 1931, and again in the first two examinations of 1933.

#### REDUCTIONS IN SPLEEN RATES.

As there were no significant changes in the yearly figures of spleen rates or sizes in the protected zone up to 1933, a table to correspond with Table III

under Nagenhalli will not be reproduced. This lack of change was also true of the peripheral zone. Between the spleen rates of the two zones, however, there was always a significant difference, the protected zone having the lower rate. The difference was  $19.5 \pm 1.4$  for all the 1929 examinations; for 1930, 1931 and 1932 the differences were all about 27.0 per cent, and were in each case significantly greater than their probable errors. For the January and April 1933 examinations the spleen rate was  $19.5 \pm 2.1$  in the protected zone and  $80.0 \pm 2.5$  in the peripheral zone, a difference of  $60.5 \pm 3.3$ . Further, the 19.5 figure was significantly lower than that for any previous January or April examination, while the 80.0 per cent rate for the peripheral zone did not differ from the rates previously found in those months.

### ANOPHELINE CATCHES.

Catches of female anophelines of the dangerous species in the Mudigere area were always small both as compared to catches of these species in other stations and to catches of other anophelines in Mudigere, as a reference to Table IV of Part II will show. For any year the catch of females of these species was not above three per cent of the total catch, and, even in the months when the dangerous species were most common, the catch was not over eight per cent of the total. Further, although the catches of female *A. culicifacies* bore some relation to the larval catches of this species, catches of females of the *fluviatilis* (*listonii*) group were much smaller than the larval catches of these species would lead one to expect. It would seem that in this area the females of the latter group did not linger in houses and cattle-sheds as in other areas, consequently the catches of these species were largely a matter of chance. (The only anopheline species so far found infected in the Mudigere area is *A. fluviatilis*.)

Table VIII gives the average catches per month of the dangerous anopheline species and of all other species, for the three groups of months and for both the protected and peripheral zones.

There was a drop in the average catch of dangerous anophelines in the protected zone in the months of group I from an average per month of 20 in 1929 to an average of 10 in 1933, a 50 per cent reduction; the maximum drop was between 1930-31 and 1931-32, a reduction of 70 per cent. In the peripheral zone the maximum reduction, 79 per cent, was between the 1929 and 1930 catches for the same group of months and there was a sharp rise in average catches between 1932 and 1933. The changes in the average monthly catches from year to year were much the same for both zones, with the change occurring one year later in the protected zone than in the peripheral. As to the months of groups II and III, the average catches of dangerous anophelines were so variable in both zones that little could be determined as to differences.

Paris green control work was not carried on during November and December 1931, or from 1st July, 1932, to the end of the year. This cannot be said to have had any decided effect on the average catches in the months of

TABLE VIII\*.

*Average catch of female anophelines per month. Mudigere area.*

		Groups of months	1929-30.	1930-31.	1931-32.	1932-33.	1933.
Protected zone	Dangerous species ..	I	20	20	6	14	10
		II	2	6	4	2	..
		III	12	2	3	5	..
	All other species ..	I	99	52	47	81	115
		II	283	157	139	261	..
		III	155	131	185	342	..
Peripheral zone	Dangerous species ..	I	33	7	12	9	20
		II	9	4	11	4	..
		III	6	3	2	8	..
	All other species ..	I	270	131	189	360	508
		II	517	328	450	652	..
		III	328	445	783	995	.

\* See Table IV for groups of months.

groups I and II in either the protected or peripheral zone. There did, however, seem to be some increase in catches in the months of group III in both zones subsequent to this cessation, and the catches of other anopheline species seemed to have risen in all three groups of months in both zones. It was not possible, however, to say that these increases were due solely to stopping the control work, since towards the end of March 1932 the control area was reduced in extent. This reduction did not seem to have affected the catches of dangerous anophelines in the months of group I in the protected zone, but may have been at least partly responsible for the rather large increase from 9 to 20 between 1932 and 1933 in the peripheral zone.

In contrast to the other areas in which paris green was used for control of anopheline breeding, it cannot be said that catches of the dangerous species in the Mudigere area demonstrated any clear-cut effect of the control work. Further, the average catches of these anophelines in the first six months of 1931 did not show any increase to account for the higher parasite rate in the protected zone, nor did the increased catches of these months in 1932 seem to result in any special outbreak of malaria. This was not so true of the peripheral zone, where the increases and decreases of average catches of dangerous

anophelines in the first six months of the various years seemed to bear some relation to the variations of the parasite rate.

During the period of observation there was a significant positive correlation between the average catch per station per month of dangerous anophelines and the parasite rates, with a one month lag of the rates, but such correlation was not found, subsequent to the beginning of control work, between catches and rates of either the protected or the peripheral zone. There was apparently some more than natural reduction in malaria in the protected zone (and, from the experience of the people of the town, a reduction in mosquitoes), although figures for catches did not clearly demonstrate this fact.

Unless the explanation for these discrepancies lies in the observations of the first paragraph of this section, and unless, in addition, the range of flight of the dangerous anophelines in this rather sparsely populated area is considerably greater than in the more populous Nagenhalli area, there does not seem to be any way of accounting for the apparent failure in adult catches. Within the controlled area, larval catches both before and after the weekly paris-greening were usually indicative of satisfactory control. When they were not so, extra paris green distribution was ordered at once.

### **III. EXPERIMENTAL CONTROL WITH PLASMOQUINE-QUININE COMPOUND (MARIKANAVE VILLAGE).**

Twelve miles up the Vedavati River from the town of Hiriyr (described in Part I) lies the village of Marikanave. It is situated at the foot of a dam across the river, and between the river bed and the channel leading from the dam sluices. The water from this channel goes back into the river bed below the village, thus keeping the river bed constantly swampy and allowing a heavy growth of reeds. Through one side of the village flows a small channel, the seepage from which also forms swampy areas. Very little of this water is used for irrigation close to the village, all paddy fields being from one-half to one mile away.

The village is divided into two parts, the one closer to the dam being the government quarters for the necessary officials. In this part of the village small ditches carry water for washing and other purposes. The main part of the village is on a small hillock about two hundred yards from the official quarters, bordering on dry land but with the channel just mentioned running through it. The whole area is within a circle with a radius of a quarter mile or less.

The population of the village, which was 449 by the records of the malaria office, is a fairly stable one, but the Government officials are subject to the usual transfers. There is no dispensary, but the village is visited once a week by the doctor in charge of the Hiriyr dispensary. Since the building of the

dam the village has been known as malarious, but no figures are available to prove its history in this respect.

Spleen and blood examinations and catches of anophelines began in this village in January 1929, and were made quarterly that year, for ten months of 1930, six months of 1931 and seven months of 1932.

### CONTROL METHODS.

The Chitaldrug District Board voted a grant of money for control experiments early in 1930. It was decided to use this money for the purchase of plasmoquine compound, in order to try its effect in malaria control when used alone. The dose was the same as that used in Nagenhalli village, and here also was given once a week only. Distribution began on 3rd April, 1930, and was continued until 11th August, 1932, when the supply of tablets was exhausted, the grant not being renewed in 1932.

In this village there was good co-operation of the people in comparison with Nagenhalli village, and it was possible to distribute tablets to an average of 76 per cent of the listed population each week. At the beginning of the work it was decided that no official pressure should be used to obtain co-operation, as it was thought that the results from such a course would be more representative of the possibilities in the usual civil population. Therefore the taking of the tablets was purely voluntary. The growing appreciation of the people was reflected in the fact that whereas for the 39 weekly distributions of 1930 the average per cent of the population taking plasmoquine was 68, this proportion rose to 80 per cent and above for the remaining 85 weekly distributions. The lowest percentage was for the second week when but 51 per cent of the people took the tablets, and the highest was 87 per cent in June 1931. As in the case of Nagenhalli, all tablets taken were swallowed in the distributor's presence and none were left for absentees.

From the records for Hiriyr town, twelve miles away, the average annual rainfall was 19.54 inches; in 1929 the rainfall was 25.51; in 1930, 27.06; in 1931, 14.26; in 1932 it was 32.11 inches. There was very little variation in these years in the maximum, minimum or mean temperatures, but the average 8 a.m. relative humidity per cent varied from 78.3 in 1929 to 72.2 in 1931.

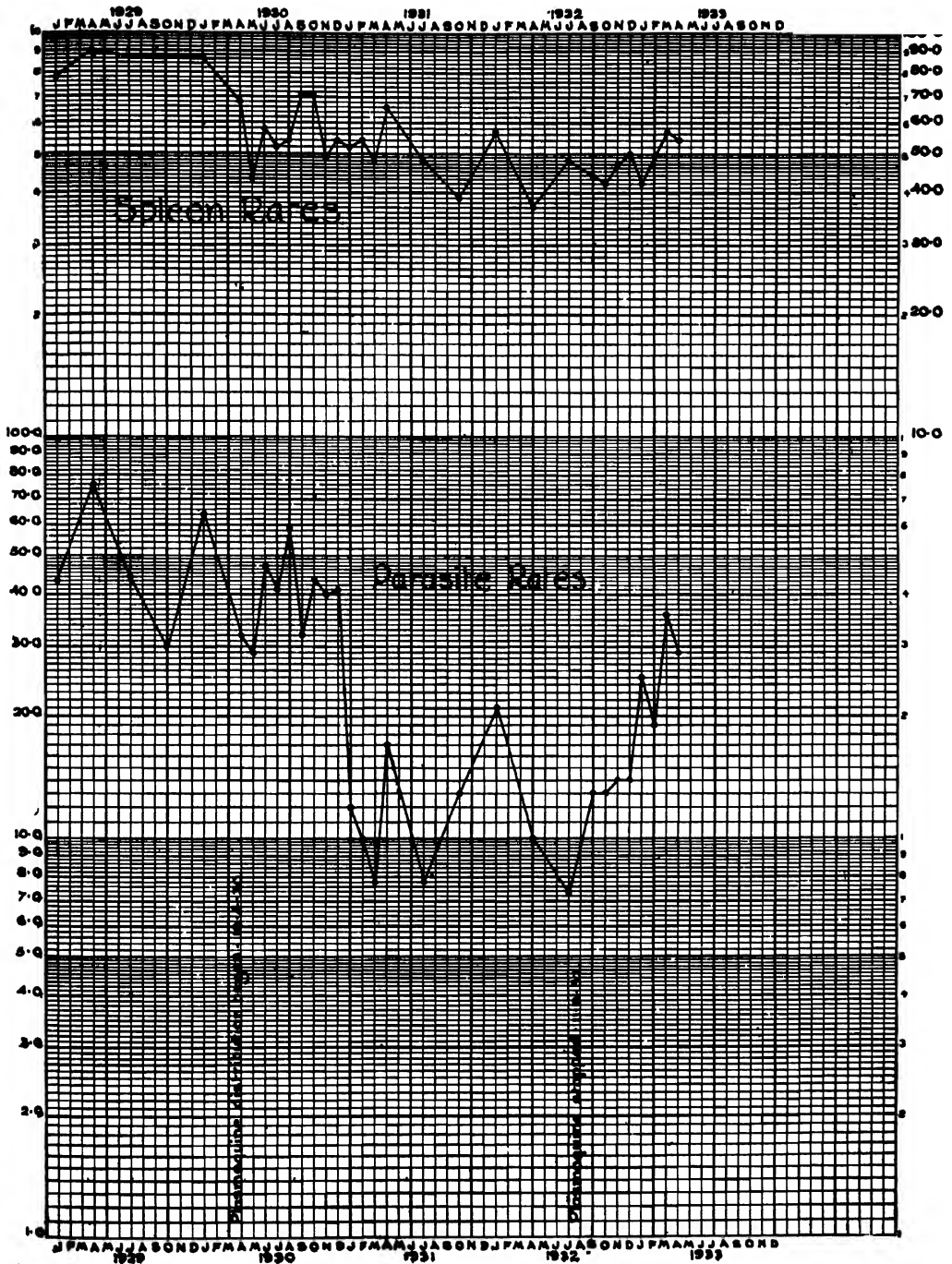
### CHANGES IN PARASITE RATES.

Graph 4 gives the parasite and spleen rates for all examinations made in Marikanave in various months of five years. For a further study of the changes in the parasite rates, Table IX gives the species and all-species rates for the four months in which examinations were made in each year. The corresponding rates for Hiriyr town have been added for comparison.

As far as climatic and other conditions are concerned it was thought that Hiriyr and Marikanave were not essentially different and that any changes in malaria from natural causes in one would also be found in the other. The

# MARIKANAVE.

GRAPH 4.





period of observation in Hiriyr extended from January 1929 to May 1931, and, on 25th May of the latter year, paris green control of anopheline breeding was begun. No decided effect of this control work could be demonstrated by the end of 1932, so it was considered that the Hiriyr rates could be used as a test for the results of the Marikanave experiment.

TABLE IX.

*Results of blood examinations made in January, April, July and October of each of four years in Marikanave and Hiriyr.*

STATIONS. Years	MARIKANAVE				HIRIYUR.			
	1929	1930	1931.	1932.	1929	1930	1931	1932
Number of persons examined.	138	121	285	353	287	194	253	316
Number of infections reported as benign tertian.	4	11	13	30	13	11	15	22
Per cent benign tertian	2.9 +1.0	9.1 ±1.8	4.6 ±0.8	8.5 ±1.0	4.5 ±0.8	5.7 ±1.1	5.9 ±1.0	7.0 ±0.9
Number malignant tertian	23	8	15	8	39	23	17	10
Per cent malignant tertian.	16.7 ±2.1	6.6 ±1.5	5.2 ±0.9	2.3 ±0.5	13.6 ±1.3	11.9 ±1.6	6.7 ±1.0	3.2 ±0.7
Number quartan ..	38	35	6	4	52	48	16	14
Per cent quartan ..	27.5 ±2.5	28.9 ±2.8	2.1 ±0.6	1.1 ±0.4	18.1 ±1.5	24.7 ±2.1	6.3 ±1.0	4.4 ±0.8
Number with any infection.*	65	56	36	42	107	84	50	47
Per cent infected ..	47.1 ±2.9	46.3 ±3.1	12.6 ±1.3	11.9 ±1.2	37.3 ±1.9	43.3 ±2.4	19.8 ±1.7	14.9 ±1.3

\*Including reported mixed infections not given elsewhere in Table.

There was no significant difference between the all-species parasite rates for the 1929 and 1930 examinations in Marikanave, but the 1931 rate was  $33.7 \pm 3.4$  below that of 1930; the rate for 1932 was not different from that for 1931, but was  $35.2 \pm 3.1$  below that for 1929. A reference to Graph 4 will show that this drop in the parasite rates was a sudden one, occurring between the examination of December 1930 and that of January 1931. The examinations made in 1933 give higher parasite rates than those for 1931 or 1932, and the rate for the combined January and April 1933 examinations is significantly higher than corresponding rates in the previous two years, but is still significantly lower than those of 1929 and 1930. The plasmoquine compound

tablets were first distributed on 3rd April, 1930, and last used on 11th August, 1932.

The same course of the all-species infection rates was observed in Hiriyyur, with the same decided drop occurring in this case between the November and December 1930 examinations, about six months before the beginning of paris green control work. In Hiriyyur, however, the drop between the 1930 and 1931 rates was  $23.5 \pm 2.9$  and that between the 1929 and 1932 rates was  $22.4 \pm 3.2$ , which is significantly smaller than the  $35.2 \pm 3.1$  drop in Marikanave. The combined January and April 1933 parasite rate in Hiriyyur was not greater than corresponding rates in 1931 and 1932, but was significantly lower than the 1929 and 1930 rates for these months.

In Marikanave the benign tertian rates were variable, that for 1930 being significantly higher than that for 1929, and the 1932 rate than that for 1931. Rates for 1930 and 1931 were not significantly different due to the larger probable error of the 1930 rate. There was a significant rise from 1929 to 1932 of  $5.6 \pm 1.4$ .

In Hiriyyur there was an increase also, although no rate was significantly different from any other. There was a check in the increase as the 1930 and 1931 rates were practically identical, whereas the increase from the 1929 to the 1930 rates and the 1931 to the 1932 rates was about the same. This check in the increase in Hiriyyur was expressed as a drop in Marikanave, for which plasmoquine may or may not have been responsible. It is not possible to draw a definite conclusion.

There was a significant decrease in the Marikanave malignant tertian rates from 1929 to 1930 of  $10.1 \pm 2.6$ , but no significant change in the rates after the latter year although they showed a steady decrease. The 1932 rate was  $14.4 \pm 2.2$  below that of 1929. For the combined January and April 1933 examinations the rate was  $10.7 \pm 1.8$ , an increase of  $7.9 \pm 2.0$  over the 1932 rate for the corresponding months. The same was true somewhat of these rates in Hiriyyur, although there the drop in rates was more gradual and no yearly rate was significantly less than the preceding one. The 1931 rate was significantly lower than the 1929 rate and the total drop between 1929 and 1932 was  $10.4 \pm 1.5$ , which was less than the drop in Marikanave but not significantly so. The January-April 1933 rate in Hiriyyur was not greater than the corresponding 1932 rate. It seems possible, therefore, that paris green in Hiriyyur and plasmoquine compound in Marikanave were responsible for the decrease in malignant tertian infections. There was no sudden drop in infections with this parasite in either Hiriyyur or Marikanave.

The sharp drop in all-species parasite rates in both Marikanave and in Hiriyyur was due to a drop in quartan infections, which took place between the December 1930 and January 1931 examinations in Marikanave and a month earlier in Hiriyyur, before any control work had been attempted in the latter place. There seemed to be no technical explanation for this very sudden fall in the rates, as methods of collection, preservation and staining, as well as the

staff, remained the same over this period, and checks of diagnoses disclosed no significant errors. There would seem to be no explanation, on the other hand, for a natural falling off of quartan infections between two monthly visits. Blood examinations in a village near Marikanave which were made in June 1932 and again a year later showed no quartan infections at all.

It is unfortunately not possible to re-check the original 1929 and 1930 blood slides. The January-April quartan rates in Hiriyr from 1929 to 1933 were as follows:  $11.6 \pm 1.8$ ,  $32.3 \pm 3.1$ ,  $5.7 \pm 1.3$ ,  $8.3 \pm 1.6$  and  $2.9 \pm 0.9$ . Some variation is shown in these rates, but in Marikanave they have remained between 1.1 and 1.8 per cent since the high rate of 1930, and did not increase in 1933 after plasmoquine distribution had ceased. The entire decrease in Marikanave from 1929 to 1932 amounted to  $26.4 \pm 2.5$  and, in Hiriyr,  $13.7 \pm 1.7$ , the difference between these two drops being significant. It is not possible to conclude that either the plasmoquine at Marikanave or the paris green at Hiriyr had anything to do with this change in quartan infections. The quartan rate in Marikanave in July 1933 was about 11 per cent.

#### CHANGES IN SPLEEN RATES.

Graph 4 gives the spleen rates as found in all examinations made in Marikanave, and Table X gives the rates for the four months in which examinations were made each year in both Marikanave and Hiriyr.

TABLE X.

*Spleen rates for examinations of January, April, July and October of each year in Marikanave and Hiriyr.*

Years.	MARIKANAVE.			HIRIYUR.		
	Number of persons examined.	Number with palpable spleen.	Per cent with palpable spleen.	Number of persons examined.	Number with palpable spleen.	Per cent with palpable spleen.
1929 ..	137	115	$83.9 \pm 2.1$	287	140	$48.8 \pm 0.0$
1930 ..	121	85	$70.2 \pm 2.8$	194	96	$49.5 \pm 2.4$
1931 .	234	123	$53.0 \pm 2.2$	253	103	$40.7 \pm 2.1$
1932 ..	294	137	$46.6 \pm 2.0$	314	122	$38.9 \pm 1.9$

In Marikanave the 1930 spleen rate was significantly lower than that for 1929 and the 1931 rate than that for 1930, while the 1931 and 1932 rates did not differ significantly. In Hiriyr, however, no spleen rate was significantly different from the preceding one, in this Table. While the total decrease in the Marikanave rates between 1929 and 1932 was  $37.3 \pm 2.9$ , in Hiriyr it was

only  $9.9 \pm 2.8$ , therefore the drop in Marikanave was significantly greater than was the case in Hiriyr. The January-April 1933 spleen rate for Marikanave was numerically, though not significantly, greater than the corresponding 1932 rate, and the graph seems to indicate a rise in the Marikanave rates for 1933, but the Hiriyr rate for January-April 1933 was significantly lower than that for 1932.

It would seem possible to conclude that plasmoquine compound in the dosage employed at Marikanave resulted in slowing down a natural increase in benign tertian infections, in markedly reducing malignant tertian infections and in considerably decreasing the spleen rates. The effect of the control work on the general parasite rate was so confused by the great change in quartan infections that any conclusions on this point are difficult, although it seemed that at least a part of the decline in these rates could be attributed to the control measures. The fact that the parasite rates started to rise almost as soon as the plasmoquine distribution was stopped, and that the January-April 1933 rates were significantly higher than in 1930 or 1931, would seem to be more than a mere coincidence.

#### CATCHES OF DANGEROUS ANOPHELINES.

Six anopheline catching stations were selected in Marikanave and catches were made whenever the village was visited for blood and spleen examinations. The average catch per station per month in the four months of Tables IX and X, for the years 1929 to 1932, was 10, 15, 19 and 11. The 1933 catches seem to indicate that they will average about the same as the 1931 figure. As no larval or adult control of anophelines was attempted, these average catches were what might have been expected. There was no drop in catches which might have explained the decrease in malaria.

Two points are of interest in this connection, in which catches at Marikanave differed from those in the other two areas discussed. About 71 per cent of the total catch were identified as belonging to the dangerous species, and *A. stephensi* constituted about 36 per cent of the catch of dangerous anophelines. *A. stephensi* in this area did not breed in wells and similar collections of water, as was the case in the cities and other areas surveyed in Mysore State, but was found breeding in swamps with slowly moving water and in the heavily reeded river bed.

#### SUMMARY.

This part of the report deals with the results of three experiments in the control of malaria in Mysore State by means of paris green and plasmoquine compound. The paris green was used in a one to three per cent mixture with road dust and ash, and was either sprayed from a hand blower or distributed by hand once a week on each breeding place of the dangerous anopheline species, with the exception of paddy fields. The plasmoquine compound was

in tablets of 1/12 grain plasmoquine and one grain quinine, of which children below ten received one and others two tablets once a week.

Each area had had a period of observation before control work began and was divided into a protected zone and a partially protected contiguous peripheral zone. Where possible a test zone was established far enough away to be unaffected by any control work, and any conclusions drawn as to the effect of the control work were checked by comparisons of changes in these zones. Spleen and blood examinations were made once a quarter at least, and anopheline catches in selected stations once a week.

In Nagenhalli both methods of control were used, although it was not found possible to get more than an average of 26.0 per cent of the population to take the plasmoquine tablets each week. The control work here resulted in a marked reduction in both spleen and parasite rates, the reduction being most noticeable about 18 or 19 months after control measures were begun. The reduction was most marked in benign tertian infections, less marked in malignant tertian infections, and least marked in quartan infections. There was about 70 per cent reduction in the average catch per station per month of females of the dangerous anopheline species and but little reduction in that of other anopheline species.

In the Mudigere area where paris green control only was attempted, there was some natural decrease in malaria, but this decrease was continued and accentuated in the protected zone only, in spite of a recurrence of malaria in this zone due to some loss of control early in 1931. The anopheline catches were confusing in this area and, for some unknown reason, were not representative as they were in the Nagenhalli area. Larval catches were satisfactory.

The distribution of plasmoquine tablets alone was used for control in Marikanave and, in spite of a very large natural drop in quartan infections, it seemed possible to conclude that the weekly distribution of the tablets had resulted in slowing down a naturally increasing benign tertian infection, in markedly reducing the malignant tertian infections, and in significantly lowering the spleen rate. Both the parasite and spleen rates gave evidence of an increase in the seven examinations made after the plasmoquine distribution was stopped.

# MIXED INFECTIONS IN THE MALARIA OF THE LOWER MONKEYS.

## Part I.

### MIXED INFECTIONS AS THE CAUSE OF APPARENT VARIATIONS IN THE MORPHOLOGY AND PATHOGENICITY OF SIMIAN PLASMODIA.

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## INTRODUCTION.

UNTIL comparatively recent years several leading malariologists held the view that the human malaria parasites might vary markedly in their morphology and in the duration of their cycle of schizogony, under certain undetermined conditions. These workers believed in the specific unicity of these parasites, and had the support of such an eminent authority as Laveran in this theory. One of the main arguments in favour of the 'unicity theory' was that a patient, who had a proven infection with one form of parasite, often showed an entirely different one during subsequent relapses. Thus a patient, who originally suffered from an undoubted infection with *P. falciparum*, frequently relapsed with an infection of *P. vivax*. The common occurrence of such a phenomenon during and after the Great War gave a great impetus to the unicity theory.

However, chiefly as the result of the work on experimental malaria in connection with the therapy of mental diseases, this view has been discarded. It has been shown that each of the recognized species of human *Plasmodium* continues true to type in both the human and the insect hosts.

It is, however, still believed by some workers that certain less striking changes, in the generally accepted morphology of these parasites, may occur inside the species. These variations have been attributed chiefly to alterations in the immunity reactions of the human host. Thus the forms described as *P. tenue* have been explained by some authors as the result of varying reactions of this nature in infections with *P. falciparum*.

Although the stability, within certain limits, of the morphological characters of the three commonly recognized Plasmodia of man is almost universally admitted, it is otherwise in the case of the Plasmodia of the lower monkeys. Several of the classical papers on the simian Plasmodia contain records of what are apparently considerable variations in the morphology and pathogenicity of these parasites. These were reported to occur in what were thought to be not only the same species, but even the same strain of parasite. One has only to study the very varied descriptions that have been given of a parasite like '*P. kochi*', to appreciate how very diverse are the morphological characters which have been attributed to a single species of *Plasmodium* (*vide* Sinton and Mulligan, 1932, 1933a).

Berenberg-Gossler (1909) reported differences in the morphology of '*P. kochi*', according to whether the infections were natural or due to artificial inoculations. This worker, as well as Gonder and Rodenwaldt (1910) and Blanchard and Langeron (1913), considers that splenectomy of the simian host caused a change in the morphology of the different Plasmodia studied by him.

More recently Grigorieva (1929) reported natural malarial infections in a specimen of *Cercopithecus fuliginosus* and in one of *Papio sphinx*. This author suggests that the differences, observed in the parasites in these two infections, may be due to changes produced in the same species of *Plasmodium* by the reactions of the bodies of hosts of different genera.

The work of Knowles and Das Gupta (1932) has given considerable support to the view that very marked changes in the morphology and pathogenicity of the same species of *Plasmodium*, may be produced by transmission of the infection to hosts of different species or genera. These workers have studied in great detail a malarial infection occurring naturally in *Silcnus irus* (*Macacus cynomolgus*). The malarial infection was conveyed by blood inoculation not only to other specimens of *S. irus*, but also to *S. rhesus*, *S. sinicus* (*M. radiatus*), *Pygathrix entellus* (*Semnopithecus entellus*), *Hylobates hoolock* (a gibbon) and to man. As a result of these experiments they state:—'We are faced with what we believe to be an entirely new problem in protozoology, if not in parasitology generally. Analogies are dangerous, and for the time being we may stick to the genus *Plasmodium*. But, given a single species of *Plasmodium*,



inoculated into and 'taking' in hosts of different genera and species in the suborder Anthropoidea, we see that in such hosts there occur very great differences in (i) susceptibility or resistance to infection, associated with (ii) great differences in the morphology of the parasite itself'.

Recent work on the American monkey parasite, *P. brasilianum*, appears to support this view. Taliaferro (1932) considers that the tertian-like parasites described by Clark (1930, 1931) in monkeys of the genus *Ateles*, and the quartan-like parasites found by the latter author in monkeys of the genus *Cebus*, are identical. Unfortunately, full details of the experiments upon which this opinion is founded do not yet appear to have been published.

It is well known that great variations may occur in the resistance or susceptibility of hosts of different genera or species to infection with some of the pathogenic protozoa. On the other hand the variations of morphology reported in some of the monkey Plasmodia are, in several instances, very startling and unexpected. If the suggested interpretation of these findings were proved to be correct, doubt might be cast upon the specific identity of many of the well-recognized pathogenic protozoa. The old question of the unicity of the human malaria parasites would require to be resurrected and reconsidered in the light of this later knowledge. The hypothesis is so revolutionary and so contrary to established ideas, that it appears to us that it must be fully tested and confirmed from every point of view, before it can be accepted as proven.

The fact that the main evidence in support of the 'unicity theory' of the human Plasmodia has been proved to be due to unrecognized mixed infections, naturally suggests a similar solution for the apparent variability of the monkey parasites. The only workers who appear to have considered such a possibility are Knowles and Das Gupta (1932), but they were of opinion that such a solution was untenable upon the evidence then available.

In our study of the Oriental Plasmodia of the lower monkeys, we have been more fortunate than many of the earlier workers, in having an excellent and varied supply of material available for study. It has become possible to re-examine the evidence in the light of fresh experimental findings. We have had at our disposal not only the original strain of infection described by Knowles and Das Gupta (1932), but also strains from natural infections in 5 other specimens of *S. irus*. Certain observations, made during an earlier study of these parasites, led us to believe that the evidence available was insufficient to exclude absolutely the occurrence of mixed infections (*vide* Sinton and Mulligan, 1933a). A series of experiments was, therefore, carried out in an attempt to elucidate this problem. The details of these are given in this paper. Having arrived at certain conclusions as the result of these experiments, the evidence, put forward by other workers in favour of the variability of different simian species of *Plasmodium*, was examined in the light of these findings. This discussion forms the subject of a second paper (Sinton and Mulligan, 1933b).

## IS A MIXED INFECTION RESPONSIBLE FOR THE VARIATIONS OF MORPHOLOGY AND PATHOGENICITY RECORDED IN A *PLASMODIUM* FOUND AS A NATURAL INFECTION OF *SILENUS IRUS*?

Knowles and Das Gupta (1932) described a *Plasmodium* from the blood of a naturally infected monkey, said to have been imported from Singapore. This animal was identified for these workers as *Cercopithecus pygerythrus*, an African species.\* The infection in the original monkey was extremely scanty, so that 'it was at first difficult to make sure that the infection was a *Plasmodium* and neither a piroplasma nor an artefact'. These workers inoculated 5 monkeys of the same species (*S. irus*) from this infected animal. In none of these was any change in the morphology of the parasite recorded and the clinical manifestations were very mild or absent. It is stated that 'both from the clinical and morphological aspects the infection in *Cercopithecus pygerythrus*\* closely resembles chronic benign tertian malaria in man'. This 'tertian' morphology they refer to as the 'Cercopithecus type'.

On the other hand Knowles and Das Gupta (1932) noted that, when the infection was inoculated into *S. rhesus*, the resultant disease was extremely severe, and always fatal if untreated. The morphology of the parasite in this species of monkey was also observed to be 'completely different from the conditions in *C. pygerythrus*,\* just as there is an extreme difference in the clinical aspects of the disease in the two different hosts'. The parasites in *S. rhesus* resembled in some respects *P. falciparum* and in others *P. malariae*. This form is referred to by Knowles and Das Gupta as the 'rhesus type', and was also found to predominate in inoculation infections in *S. sinicus*, *Py. entellus*, *H. hoolock* and man.

Knowles and Das Gupta (1932) concluded that they were dealing with a single species of *Plasmodium*, and that this parasite changes its morphology and pathogenicity when inoculated into animals of different genera and species. They considered, however, the possibilities that their findings could be explained by assuming (a) the presence of a mixed infection in the natural host, or (b) the presence of latent infections in some of the animals into which sub-inoculations had been made. For reasons which will be given in detail later, they decided that these explanations were untenable.

Through the kindness of the above-mentioned workers, we obtained a specimen of *S. irus* (*C. pygerythrus*) inoculated with the strain of malarial infection with which they had made their experiments. We have been able to confirm, from our personal observations, the same *apparent* changes in the morphology and pathogenicity recorded by Knowles and Das Gupta (1932), after passage of this strain to healthy specimens of *S. rhesus*.

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\* In a subsequent communication (Knowles, 1932b), this identification is corrected to *Silenus irus* (*Macacus cynomolgus*), an Oriental species of monkey.

Subsequently we were successful in obtaining 5 other specimens of *S. irus*\* showing natural malarial infections. Like the original host of the strain used by Knowles and Das Gupta, these animals were bought in Calcutta and were said to have been imported from Singapore.

The parasites in these natural infections were very scanty, but an extremely severe infection was produced when blood from any of them was inoculated into normal specimens of *S. rhesus*. The morphology of the Plasmodium, observed in the latter species of monkey under these conditions, corresponded exactly with the 'rhesus type' described by Knowles and Das Gupta (1932) (vide infra *P. knowlesi*). Some differences were, however, noted in the morphology of the parasites in some of the natural infections, and also in some of the specimens of *S. irus* inoculated from these hosts. Although, in most instances, the predominant form of parasite corresponded morphologically with the 'Cercopithecus type' of Knowles and Das Gupta (vide infra *P. inui* var. *cynomolgi*), careful search revealed forms, usually very scanty, which resembled more closely the 'rhesus type'. The latter forms are probably those mentioned by Knowles (1932a) as resembling *P. malariae*.

These findings suggested very strongly that one was dealing with mixed infections, in some instances at least. However, in view of the findings and statements of Knowles and Das Gupta, we were prepared, at first, to accept their interpretation, i.e., that marked changes of morphology could occur in a single species of monkey *Plasmodium*, when this was inoculated into animals of a different genus or species from the original host. Our earlier observations appeared to confirm this idea and no conclusive evidence to the contrary was available. Such a view was also supported to some extent by the statements of Taliaferro (1932) in respect to the malarial infections of Panamanian monkeys, and by the suggestions of Grigorieva (1929) about African Plasmodia.

When it became known that the so-called '*Cercopithecus pygerythrus*' was not an African monkey, but an Oriental one of the genus *Silenus* (*S. irus*), the view that the apparent changes could be attributed primarily to infection in a different genus of monkey became untenable. Our suspicions about the possibility of mixed infections were naturally re-awakened, and the problem required further consideration. The only support for the theory of mixed infections was our earlier observation that in some instances small numbers of parasites, corresponding more closely to the 'rhesus type', had been found in both naturally infected and inoculated specimens of *S. irus*.

The first obvious step in the investigation was to make a careful study of the various morphological appearances shown by the parasites in infections of

\*Through the kindness of Captain P. J. Barraud, F.R.S., and the Bombay Natural History Society, the identity of this monkey has been confirmed at the British Museum by R. I. Pocock, Esq., F.R.S. The latter authority identified a specimen sent to him as *Macacus fascicularis*, the crab-eating macaque. This name is given by Stiles and Nolan (1929) as a synonym for *Silenus irus* (*Macacus cynomolgus*).

the 'rhesus' and 'Cercopithecus' types. When this had been done, further extended observations were carried out on the bloods of the five naturally infected specimens of *S. irus*. These showed that the occurrence of parasites in the peripheral blood was intermittent. The predominant form of *Plasmodium* present at any one time was usually the 'Cercopithecus type', but on other occasions parasites of the 'rhesus type' were also detectable.

After many trials we eventually succeeded in isolating, by blood inoculation from the same naturally infected specimen of *S. irus*, two distinct types of *Plasmodium* in *S. rhesus*. One of these types was identical to all appearances with that usually seen in *S. rhesus* (the 'rhesus type'), while the other was indistinguishable from the form which is usually predominant in *S. irus* (the 'Cercopithecus type'). The original host, from which these two distinct types were isolated, had a parasitic infection apparently similar in all respects with that observed in other specimens of *S. irus* infected in nature. No differences could be detected between this infection and that in the infected specimen of *S. irus* kindly sent us by Colonel Knowles. It also corresponded very closely with the descriptions given by Knowles and Das Gupta (1932) of infections in this species of monkey (the 'Cercopithecus type').

These findings considerably strengthened our original impression that the natural infections studied by us might be mixed ones, and that the marked changes of morphology, which appeared to occur in the parasites, might be due to an undetected infection of this nature. Two apparently distinct species of monkey *Plasmodium* had now been isolated, one of which was morphologically indistinguishable from *P. inui* var. *cynomolgi* Mayer, 1907 (the 'Cercopithecus type'). The other (the 'rhesus type') was apparently a new species, for which the name *P. knowlesi* was proposed by Sinton and Mulligan (1932).

It was now necessary to consider—

- (a) whether these two apparently distinct species of *Plasmodium* were merely morphological variations of the same parasite due to passage into different species of monkey,
- (b) whether, if this did not prove to be the case, the occurrence of an undetected latent infection in the inoculated animal, *S. rhesus*, might not be responsible for the presence of the two different species of parasite, or
- (c) whether, if the two previous suggestions were untenable, a mixed infection in the original host would account for the very varied morphological and pathogenic changes which appeared to occur, without having to rely for an explanation upon a new conception in protozoology.

A preliminary account of some of the experiments made to elucidate these problems has already been given in an appendix to a previous paper (Sinton and Mulligan, 1933a). It is proposed in the present article to give fuller details of these and later investigations.

# I. ARE *P. KNOWLESI* AND *P. INUI* VAR. *CYNOMOLGI* TWO DISTINCT SPECIES ?

The investigations carried out to decide this question may be summarized as follows :—

- A. A detailed study and comparison of the morphological and other characters of the two types of parasite.
- B. Experiments to determine whether pure strains of these parasites remained true to type, after passage and sub-passage through various species of monkey.
- C. Observations on the morphology of each type of parasite under varying conditions of immunity in the animal host.
- D. Experiments to observe whether passage of the parasites through the insect host caused any change in their diagnostic characteristics.
- E. A comparison of the pathogenic effects produced by each type of parasite in the same species of monkey.

## A. STUDY OF THE MORPHOLOGY OF THE TWO PARASITES.

When the two apparently distinct species of monkey *Plasmodium* referred to above were originally isolated in *S. rhesus*, each was subjected to very careful study. This was necessary to determine what morphological and other characteristics would serve to differentiate one from the other. The chief diagnostic features, by which these two parasites may be distinguished, are summarized in Table I. A more detailed account of these characters has been given elsewhere (Sinton and Mulligan, 1933a).

TABLE I.

Summary of the chief characters by which *P. knowlesi* and *P. inui* var *cynomolgi* may be differentiated.

<i>P. knowlesi</i> [the 'rhesus' type of Knowles and Das Gupta (1932)].	<i>P. inui</i> var <i>cynomolgi</i> [the 'Cercopithecus' type of Knowles and Das Gupta (1932)]
1. Young rings one-fourth to one-half diameter red cell; usually show thickening of protoplasm opposite chromatin.	1 Young rings one-fifth to one-third diameter red cell; usually thin and hair-like.
2. Slightly older forms occur as rings with more solid appearance.	2 Slightly older forms often very irregular
3. Older trophozoites rounded up, or only very slightly amoeboid; vacuole very inconspicuous or absent.	3 Older trophozoites amoeboid or very amoeboid; vacuole usually very conspicuous.
4. Mature schizonts size of, or smaller than, normal red cell; number of merozoites commonly about 10.	4. Mature schizonts usually larger than normal red cell; number of merozoites usually about 16.

*P. knowlesi*  
[the 'rhesus' type of Knowles and  
Das Gupta (1932)].

*P. inui* var. *cynomolgi*  
[the 'Cercopithecus' type of Knowles  
and Das Gupta (1932)].

- |   |   |
|---|---|
| <p>5. Pigment appears early; relatively abundant; granules fairly coarse, and vary from greenish brown to almost black.</p>   | <p>5. Pigment appears later; relatively scanty; granules finer and of golden brown colour.</p>  |
| <p>6. Infested red cells not appreciably enlarged; often show characteristic distortion—oval, fimbriated, crenated, polyhedral, etc.; stippling usually absent with ordinary stains, but demonstrable with larger forms by special stains, and often giving cell a mottled reddish pink appearance.</p> | <p>6. Infested red cells usually enlarged; stippling like Schüffner's dots easily demonstrable by ordinary stains; appears early and is constant, except with youngest forms.</p> |
| <p>7. Gametocytes like those of <i>P. malariae</i>; about size of normal red cell; pigment coarse and dark.</p>   | <p>7. Gametocytes like those of <i>P. vivax</i>; usually larger than normal red cell; pigment finer and lighter in colour.</p>  |
| <p>8. Duration of schizogony cycle—24 hours.</p>  | <p>8. Duration of schizogony cycle—48 hours.</p>  |
| <p>9. Pathogenicity. In our experience, inoculation infections in <i>S. rhesus</i> almost invariably fatal unless treated in primary attack.</p>  | <p>9. Pathogenicity. In our experience, inoculation infections in <i>S. rhesus</i> invariably recover spontaneously.</p>  |

The very marked contrast between the morphological and other characters of these two parasites, suggests strongly that they are two distinct species. Numerous careful observations, made on a considerable number of monkeys of different species, have shown conclusively that the duration of the schizogony cycle is 24 hours in one, and 48 hours in the other (*vide* Sinton and Mulligan, 1933a).

The duration of this cycle in each has been found to remain unaltered, irrespective of the species of animal host in which the infection is observed. While it might be accepted that minor morphological changes could occur, such a fundamental difference, as a complete alteration in the duration of the schizogony cycle, would scarcely be credible in the same species of *Plasmodium* in the same species of monkey host.

### Conclusions.

(a) Two apparently distinct species of *Plasmodium* have been isolated in *S. rhesus*, inoculated from the same naturally infected specimen of *S. irus*.

(b) One of these species corresponds to the 'Cercopithecus type' described by Knowles and Das Gupta (1932), and the other to the 'rhesus type' described by these authors.

(c) The former has been identified as *P. inui* var. *cynomolgi* Mayer, 1907, and the latter as *P. knowlesi* Sinton and Mulligan, 1932.

(d) These two species of monkey *Plasmodium* differ markedly in their morphological characteristics, and in the duration of their schizogony cycles.

#### B. RESULT OF ANIMAL PASSAGE UPON THE MORPHOLOGY OF THE TWO TYPES OF PARASITE.

The findings recorded above supported our impression that the two types of parasites studied by us were two distinct species of *Plasmodium*. The original suspicion that the infections observed in naturally infected monkeys were mixed ones was also strengthened. It was necessary, however, to confirm this, and to rule out the possibility that very marked differences in the morphology and schizogony cycle of the parasites could be brought about by the influence of factors connected with the animal host.

If such marked variations in the behaviour of a single species of *Plasmodium* were possible, when observed in different species of monkey, it would be reasonable to expect that a reversion to the original type of morphology would occur when the infection was re-inoculated into animals of the same species as the original host. Indeed it would not be surprising if, in the course of passages through different genera and species of monkeys, many forms were encountered intermediate between the two distinct species described.

On the other hand, if these two types of parasites represented two distinct species of *Plasmodium*, there would be every reason to anticipate that each would remain true to type under all such conditions.

As the result of experiments conducted along these lines, it has been possible to show that *P. knowlesi* and *P. inui* var. *cynomolgi* are two distinct species, and that each remains true to type after passage through monkeys of different species. The experimental work on which this conclusion is founded is detailed below.

#### 1. The effects of animal passage upon the morphology and schizogony cycle of *P. knowlesi* Sinton and Mulligan, 1932.

(a) *Results of passage of P. knowlesi, by blood-inoculation, through different species of monkey, to determine the stability of its diagnostic characters.\**

(i) Passage of *P. knowlesi* to normal specimens of *S. rhesus*.

The monkeys employed in these experiments were young specimens of *S. rhesus* captured in nature in northern India. There is good reason to believe that such monkeys are always free from plasmodial infections in nature, or if these occur, they must be extremely rare (*vide infra*)\*.

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\* In recording the results of these experiments, the term 'diagnostic characters' is used to denote not only the morphological features of the parasite, but also the changes in the infested red blood cells, and the duration of the cycle of schizogony.

**Experiment (1).**

In the course of our investigations into monkey malaria, we have had occasion to observe five different strains of *P. knowlesi* after passage through *S. rhesus*. Over 100 observations of this nature have been made, and as many as 14 serial sub-passages of a single strain through *S. rhesus* have been carried out.

**Result.**—In every instance the diagnostic characters of *P. knowlesi* have remained constant and true to type, when a pure strain was used. This result was observed irrespective of whether the passages were made from animals in the acute or chronic stages of infection.

(ii) Passage of *P. knowlesi* to normal specimens of *S. irus*.

The difficulty and expense of obtaining specimens of *S. irus* in northern India, and the further difficulty of excluding the possibility of latent malarial infections in imported animals, prevented extensive investigations along these lines. Two specimens of *S. irus*, believed to be free from plasmodial infection,\* were selected. Each of these was inoculated with a strain of *P. knowlesi* which had remained pure after repeated passage through *S. rhesus*.

**Experiment (2). Monkey No. 103 (*S. irus*).**

This monkey was inoculated with a pure strain of *P. knowlesi* which had been isolated in *S. rhesus*. Parasites appeared on 7th day. The infection ran a mild course and parasites were never seen in large numbers. Blood films were taken at 3-hourly intervals, and these were very carefully examined. Following the initial mild attack, the infection became chronic and parasites were observed in the peripheral blood for a month, but later disappeared. The blood of this monkey was inoculated into a normal specimen of *S. rhesus* 75 days after parasites were last seen. The latter animal developed an acute infection with typical *P. knowlesi*.

**Result.**—At no stage in the course of this prolonged experiment could any change be detected in the morphology, nor in the duration of the schizogony cycle, of this parasite. It appeared to remain, at all times, identical with the 'rhesus type' described by Knowles and Das Gupta (1932) in *S. rhesus*, and not with the 'Cercopithecus type' recorded by them in *S. irus*.

**Experiment (3). Monkey No. 121 (*S. irus*).**

This monkey was inoculated from a specimen of *S. rhesus* infected with a pure strain of *P. knowlesi*. Parasites appeared on 8th day, and a mild attack developed.

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\*It will be seen from a later paper (Sinton and Mulligan, 1933b; Appendix) that natural malarial infections, often of a latent character, are common in this species of monkey. For this reason we have regarded all imported animals of this species as likely to be infected. The routine procedure used to detect such latent infections has been described later in this paper (Appendix I). Any animal, in which a careful application of these methods failed to reveal a plasmodial infection, was considered to be free from malaria for the purposes of the experimental work recorded here.



The infection was observed for a period of 6 days after which this monkey was superinfected with a pure strain of *P. inui* var. *cynomolgi* [vide experiment (26)].

*Result.*—The morphology of *P. knowlesi* in *S. irus* was similar in all respects to that observed in *S. rhesus*.

(iii) Passage of *P. knowlesi* to a normal specimen of *S. sinicus*.

Experiment (4). Monkey No. 82 (*S. sinicus*).

This monkey, previously found to be free from malarial infection, was inoculated with a pure strain of *P. knowlesi*. Parasites appeared on 5th day, and an acute attack, associated with constitutional symptoms, developed. Blood films were taken at 3-hourly intervals during the acute phase. Quinine treatment, given on two consecutive days, was sufficient to control the primary attack, and subsequently no relapses occurred.

*Result.*—A careful study of the blood films taken during the acute attack showed that the diagnostic features of *P. knowlesi* remained unaltered after passage to *S. sinicus*. Similarly no changes were seen during the ensuing chronic infection, which was observed for 4 months.

(iv) Passage of *P. knowlesi* to a normal specimen of *Pygathrix schistaceus*.

Experiment (5). Monkey No. 67 (*Py. schistaceus*).

A pure strain of *P. knowlesi* was inoculated into a normal specimen of *Py. schistaceus*. A very severe infection resulted which was controlled by treatment. Blood films were taken at hourly intervals before treatment was commenced. This animal died 7 days later from an intercurrent disease.

*Result.*—A very careful study of the serial blood films, taken during the acute phase of the disease, showed that the diagnostic features of *P. knowlesi* were undistinguishable from those observed in *S. irus*, *S. rhesus* and *S. sinicus*.

### (b) Summary.

The morphological characteristics, and the duration of the schizogony cycle of pure strains of *P. knowlesi*, isolated in *S. rhesus* remained unchanged when the infection was :—

- (a) Passaged and sub-passaged by blood inoculation through a large number of specimens of *S. rhesus*.
- (b) Passaged to either *S. irus* or *S. sinicus*.
- (c) Re-passaged from *S. irus* to *S. rhesus* and *vice versa*.
- (d) Passaged to a monkey of a different genus and species, namely, *Pygathrix schistaceus*.

No tendency for *P. knowlesi* (the 'rhesus type') to change into the 'Cercopithecus type' was observed, nor were any forms intermediate between these two types encountered in any animal.

**2. Effects of animal passage on the morphology and schizogony cycle of *P. inui* var. *cynomolgi* Mayer, 1907.**

(a) *Results of passage of P. inui var. cynomolgi, by blood inoculation, through different species of monkey, to determine the stability of its diagnostic characters.*

(i) Passage of *P. inui* var. *cynomolgi* to normal specimens of *S. rhesus*.

**Experiment (6).**

The strain of *P. inui* var. *cynomolgi*, originally isolated in pure form in *S. rhesus*, has been passaged by direct blood inoculation to 11 normal specimens of *S. rhesus*, including 3 serial sub-passages from *S. rhesus* to *S. rhesus*.

*Result.*—In every instance the morphological characters of this parasite have remained true to type. There has been no suggestion of any variation from the morphology of the strain originally isolated, nor in the duration of the schizogony cycle.

(ii) Passage of *P. inui* var. *cynomolgi* to normal specimens of *S. irus*.\*

**Experiment (7). Monkey No. 129 (*S. irus*).**

This monkey was inoculated from a specimen of *S. rhesus* infected with a pure strain of *P. inui* var. *cynomolgi*. Parasites appeared on 3rd day, and a mild infection resulted. This infection was subsequently re-passaged into a normal specimen of *S. rhesus*.

*Result.*—The diagnostic characters of *P. inui* var. *cynomolgi* remained entirely unchanged when observed in *S. irus* and when re-passaged from the latter monkey to a normal specimen of *S. rhesus*.

**Experiment (8). Monkey No. 119 (*S. irus*).**

This animal was inoculated from Monkey No. 129 [*S. irus*, *vide* experiment (7)]. Parasites were detected on 4th day after inoculation, and a mild infection resulted. This animal was subsequently observed for a period of 4 months.

*Result.*—The characters of this parasite were similar in all respects to those seen in *S. rhesus* infected with *P. inui* var. *cynomolgi*.

(iii) Passage of *P. inui* var. *cynomolgi* to a normal *S. sinicus*.

**Experiment (9). Monkey No. 115 (*S. sinicus*).**

This animal, previously found to be normal, was inoculated from a specimen of *S. rhesus* infected with a pure strain of *P. inui* var. *cynomolgi*. Parasites were detected on 11th day. A mild infection resulted. The monkey was observed for a period of 5 months.

*Result.*—The diagnostic characters of the parasite showed no change from those observed in *S. rhesus* and *S. irus*.

\* The same precautions were taken to select monkeys free from latent malarial infections, as were taken for passage experiments with *P. knowlesi* (*vide* foot-note, p. 729).

*(b) Summary.*

The morphological characteristics and the duration of the schizogony cycle of a pure strain of *P. inui* var. *cynomolgi*, isolated in *S. rhesus*, were found to remain constant

- (a) when the infection was passaged and sub-passaged by blood inoculation through a number of animals of the latter species,
- (b) when passaged to either *S. irus* or *S. sinicus*,
- (c) when re-passaged from *S. irus* to *S. rhesus* and *vice versa*.

No tendency to revert to the 'rhesus type' of *Plasmodium* could be detected under any of these conditions, nor were any forms encountered which were intermediate between the 'rhesus' and the 'Cercopithecus' types.

**3. Conclusions.**

The results of the above experiments clearly indicate that

- (a) *P. knowlesi* and *P. inui* var. *cynomolgi* are two entirely distinct species of monkey *Plasmodium*, each of which is characterized by a distinctive morphology,
- (b) the duration of the cycle of schizogony in the former species is 24 hours, and in the latter 48 hours,
- (c) these diagnostic features remain constant for each species after repeated passage and sub-passage, by blood inoculation, irrespective of the species or genus of monkey host used, and of the species from which passaged.

**C. THE DIAGNOSTIC CHARACTERISTICS OF *P. KNOWLESI* AND *P. INUI* VAR. *CYNOMOLGI* UNDER VARYING CONDITIONS OF IMMUNITY IN THE HOST.**

Some workers have suggested that the morphology of certain of the human Plasmodia may vary according to the degree of resistance or susceptibility present in the host. It was therefore necessary to study these simian parasites from this point of view. Observations were made

- (1) on infected animals at different stages during the course of their infections,
- (2) on animals which had been highly immunized by superinfection, not only with homologous, but also with heterologous, strains of the same species of parasite, and
- (3) on animals whose resistance to infection had been disturbed by removal of their spleens.

In this way it was hoped to ascertain whether changes in immunity of the animal host could be responsible for the reported variations in the diagnostic features of the two types of parasite.

**1. The diagnostic features of *P. knowlesi* and *P. inui* var. *cynomolgi* in acute and chronic infections.**

A very careful study was made of the diagnostic characteristics of each of these two parasites at all stages in the course of infection. Examinations

were made from the time of the first appearance of parasites until the infections had run a chronic course. In some instances parasites were still detectable as long as 11 months after the first inoculation. In this way it was possible to observe the behaviour of each species of parasite in animals in which little or no immunity was present, as well as in those which had had time to develop very considerable tolerance to the species of *Plasmodium* present.

Even when infections had remained latent for considerable periods, the characters of the parasites, which re-appeared after injection of foreign protein, showed no detectable differences from the type originally present.

Although observed under conditions of extreme variation in the resistance of the animal host, the diagnostic features of both *P. knowlesi* and *P. inui* var. *cynomolgi* showed no deviation from those recorded in the descriptions given above.

## **2. The influence of immunity, following superinfection, on the diagnostic characters of *P. knowlesi* and *P. inui* var. *cynomolgi*.**

A large series of superinfection experiments, with both homologous and heterologous strains of parasite, have been recorded in detail in an earlier paper (Mulligan and Sinton, 1933).

In spite of very diverse degrees of immunity present in the animal host at different stages of these experiments, it was found that each species of parasite remained, at all times, true to type. No intermediate forms between *P. knowlesi* and *P. inui* var. *cynomolgi* could be detected, nor did the duration of the schizogony cycle of either parasite show any tendency to change.

## **3. The effects of splenectomy of the animal host on the diagnostic characters of *P. knowlesi* and *P. inui* var. *cynomolgi*.**

Berenberg-Gossler (1909) and Gonder and Rodenwaldt (1910) found that, after splenectomy, the parasites in their monkeys showed peculiar division forms, which they considered to be degenerate. These changes were so striking that Berenberg-Gossler thought, at first, that he was dealing with a new species of *Plasmodium*. Blanchard and Langeron (1913) also reported that certain changes in the morphology of the parasites could be detected after splenectomy of the monkey hosts. On the other hand Bouilliez (1913) and Leger and Bouilliez (1913) did not observe any morphological changes in their parasites after splenectomy of their experimental animals.

In view of these divergent findings, it was decided to investigate whether any appreciable changes in the morphology of either *P. knowlesi* or *P. inui* var. *cynomolgi* could be detected, following splenectomy of monkeys infected with these parasites. Removal of the spleen would be expected to produce a very considerable alteration in the resistance of the animal host to such infections.

A brief summary of the experiments carried out to investigate this problem is given below. It is hoped to give more complete details of these and other experiments in a later paper.

(a) *Effects of splenectomy of the monkey host on the morphology of P. knowlesi.*

(i) Infections transmitted to splenectomized animals.

Experiment (10). Monkey No. 177 (*S. rhesus*).

This normal monkey was splenectomized, and 7 days after the operation an inoculation with a pure strain of *P. knowlesi* was given. Parasites appeared after an incubation period of 9 days, and an acute attack developed.

*Result.*—The morphology of *P. knowlesi* observed in this animal appeared to be identical with that observed after inoculation of an intact specimen of *S. rhesus*.

Experiment (11). Monkey No. 159 (*S. irus*).

This monkey, previously found to be free from malarial infection, was splenectomized. The following day an inoculation with a pure strain of *P. knowlesi* was given. Parasites appeared after an incubation period of 2 days, and an acute attack developed. Treatment was necessary to save the animal's life.

*Result.*—The morphology of the parasite observed in this animal was similar in all respects to that seen in intact specimens of *S. rhesus* and *S. irus*.

(ii) Splenectomy of infected animals.

Experiment (12). Monkey No. 100 (*S. rhesus*).

This monkey was known to have been infected with a pure strain of *P. knowlesi* 5 months previously. Splenectomy was performed 51 days after parasites had last been seen. Five days after the operation parasites re-appeared in the peripheral blood. An acute attack developed of such severity that it was considered necessary to give treatment to save the animal's life.

*Result.*—Splenectomy was followed by a relapse of considerable severity. The morphology of the parasites was identical with that previously observed in the same animal.

Experiment (13). Monkey No. 103 (*S. irus*).

This monkey had been infected with a pure strain of *P. knowlesi* 5 months previously. No parasites had been seen in the peripheral blood for 70 days before splenectomy was performed. Parasites re-appeared in the peripheral blood 8 days after the operation. A very severe attack developed from which the animal died.\*

*Result.*—Splenectomy was followed by a severe, and ultimately fatal, attack with *P. knowlesi*. The morphology of the latter parasite remained indistinguishable from that observed in this monkey in the primary attack.

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\*It is noteworthy that this animal recovered from the primary attack spontaneously. This is the first instance during the course of our investigations in which a fatal result has followed an infection with *P. knowlesi* in *S. irus*.

(b) *Effects of splenectomy of the monkey host on the morphology of P. inui var. cynomolgi.*

(i) Infections transmitted to splenectomized animals.

Experiment (14). Monkey No. 172 (*S. rhesus*).

Splenectomy was performed on this monkey which was previously known to be normal. An inoculation with a pure strain of *P. inui* var. *cynomolgi* was given 17 days after the operation. Parasites appeared in the blood 7 days later, and an attack developed comparable in severity to that usually seen in primary attacks in intact specimens of *S. rhesus*.

*Result.*—An attack of moderate severity occurred, and recovery was spontaneous. The morphology of the parasite was identical with that seen in intact animals infected with a pure strain of *P. inui* var. *cynomolgi*.

Experiment (15). Monkey No. 158 (*S. irus*).

This monkey, previously found to be free from infection, was splenectomized. An inoculation with a pure strain of *P. inui* var. *cynomolgi* was given 16 days after the operation. Parasites appeared after an incubation period of 6 days. A mild infection developed from which recovery was spontaneous.

*Result.*—The course of the infection was identical with that usually seen in intact specimens of *S. irus*. The morphology of the parasite remained unaltered.

(ii) Splenectomy of infected animals.

Experiment (16). Monkey No. 149 (*S. rhesus*).

This monkey had suffered from a chronic infection with a pure strain of *P. inui* var. *cynomolgi*. This was of two months' duration when splenectomy was performed. No parasites had been seen for 11 days prior to the operation. Parasites re-appeared 9 days after splenectomy, and an attack of moderate severity resulted. Recovery was spontaneous.

*Result.*—Splenectomy was followed by a relapse, the severity of which was similar to that of the primary attack. The morphology of the parasite was identical with that observed before splenectomy was performed.

Experiment (17). Monkey No. 138 (*S. rhesus*).

This monkey was suffering from a chronic infection with a pure strain of *P. inui* var. *cynomolgi* of 15 weeks' duration when splenectomy was performed. Parasites had been seen in the blood 3 days before the operation. A very severe attack developed on the 5th day following splenectomy, and hæmoglobinuria was observed on 8th day. The animal recovered with treatment.

*Result.*—Splenectomy was followed by a very severe attack accompanied by hæmoglobinuria.\* The morphology of the parasite was identical with that observed in the primary attack.

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\* This is the only occasion on which we have observed hæmoglobinuria during the course of a pure infection of *P. inui* var. *cynomolgi*.

(c) *Effects of splenectomy of the monkey host on the morphology of mixed infections with P. knowlesi and P. inui var. cynomolgi.*

(i) Splenectomy in natural mixed infections.

Experiment (18). Monkey No. 56 (*S. irus*).

This monkey was suffering from a mixed infection (naturally acquired) with both *P. knowlesi* and *P. inui* var. *cynomolgi* when splenectomy was performed. No parasites had been observed for 8 days prior to the operation. Parasites re-appeared 1 day later. Both species of *Plasmodium* were seen, but *P. knowlesi* markedly predominated. An attack of considerable severity resulted and treatment was required to arrest the progress of the disease.

*Result.*—Both *P. knowlesi* and *P. inui* var. *cynomolgi* were identified after splenectomy. The morphological characters of both species appeared to be identical with the forms seen in this monkey before removal of the spleen.

(ii) Splenectomy in experimental mixed infections.

Experiment (19). Monkey No. 121 (*S. irus*).

This monkey had previously suffered from an experimentally produced mixed infection with both *P. knowlesi* and *P. inui* var. *cynomolgi* when splenectomy was performed. At the time of operation the infection was of 4 months' standing, and parasites had been seen 38 days previously. *P. knowlesi* re-appeared 3 days after removal of the spleen, and an acute attack developed from which recovery was spontaneous.

*Result.*—Splenectomy was followed by an attack with *P. knowlesi* of appreciably greater severity than that of the primary attack. The morphological characters of *P. knowlesi* remained identical with those observed in the same animal before operation.

Experiment (20). Monkey No. 154 (*S. rhesus*).

This monkey was suffering from an experimental mixed infection of 7 weeks' duration when splenectomy was performed. At the time of operation *P. inui* var. *cynomolgi* was the only detectable parasite, and it remained so for 3 days after the spleen was removed. *P. knowlesi* then appeared and rapidly became the predominant infection. An acute attack with the latter parasite developed, and treatment was considered necessary to save the animal.

*Result.*—Both *P. knowlesi* and *P. inui* var. *cynomolgi* were distinguishable after splenectomy. The former parasite quickly became the predominant infection. The morphological characters of both parasites remained identical with those observed before removal of the spleen.

(d) *Summary.*

Under the conditions of our experiments, no changes could be detected in the diagnostic characters of either *P. knowlesi* or of *P. inui* var. *cynomolgi*, as the result of splenectomy of the animal host. These results were obtained irrespective of whether the infection was transmitted to the hosts after splenectomy, or whether the operation was performed upon animals which were already infected.

#### 4. Conclusions.

No change in the morphological characters, or in the duration of the schizogony cycle, could be detected in pure infections with either *P. knowlesi* or *P. inui* var. *cynomolgi* under the following conditions:—

(a) When the infection resulted from a passage made from an acute primary attack, or from an old chronic infection, in either *S. rhesus* or *S. irus*.

(b) When the parasites were studied in fresh infections, or at any time during a chronic course lasting as long as 11 months.

(c) When the animals had been hyper-immunized by superinfection with homologous or heterologous strains.

(d) When the immunity of the animal hosts had been disturbed by removal of the spleen.

#### D. THE INFLUENCE OF MOSQUITO-PASSAGE ON THE DIAGNOSTIC CHARACTERS OF *P. KNOWLESI* AND *P. INUI* VAR. *CYNOMOLGI*.

It was considered that a study of the characters of these two types of parasite after insect transmission might be of assistance in confirming their specific identity. If, after passage through the mosquito, the diagnostic features of these two types of parasite remained unchanged, it would be strong evidence that each was a true and distinct species of monkey *Plasmodium*.

#### 1. Results of attempts to transmit *P. knowlesi* to healthy monkeys by passage through the mosquito.

##### Experiment (21).

Very considerable difficulty has been experienced in obtaining suitable infected animals for mosquito-feeding experiments. On account of the extreme virulence of *P. knowlesi* for *S. rhesus*, early treatment is necessary to save the animal's life. Although gametocytes may be encountered before treatment is commenced, these are not usually abundant and it is doubtful whether they are infective to mosquitoes at this early stage. In treated animals gametocytes are usually comparatively scanty. The occurrence of suitable gametocyte carriers is, therefore, much less frequent than is the case with animals infected with *P. inui* var. *cynomolgi*. In the latter infection no treatment is required, and the disease may be allowed to run its natural course without danger to the life of the animal host. Feeding experiments have, however, been carried out on a number of monkeys infected with *P. knowlesi*. Up to the present, all attempts have been made on *S. rhesus* monkeys experimentally infected, because no suitable naturally infected animals have been available. The development of the sporogony cycle up to the formation of sporozoites in the salivary glands of *A. annularis* (*A. fuliginosus*) has been demonstrated on several occasions. The percentage of mosquitoes which became infected after feeding on infected animals was, however, very small, and in most cases completely negative results were obtained.

It is possible that the difficulty experienced in infecting mosquitoes with *P. knowlesi* may be attributable to the unsuitability of the local species of anopheline mosquitoes used, rather than to unfavourable conditions in the animal host.

**Result.**—Up to the time of writing it has not been found possible to transmit *P. knowlesi* to healthy monkeys through the bites of infected



mosquitoes. It was, therefore, impossible to determine whether the morphology of this parasite remained true to type after insect transmission. Knowles (1932a) apparently experienced similar difficulty in infecting mosquitoes with this parasite.\* Further experiments are now in progress to settle this question.

As there is no reason to suspect that the morphology of *P. knowlesi* should change after insect transmission, and as no worker appears to have claimed that variations in the morphology of any Plasmodium occur after passage through the mosquito or other insect, the absence of this proof seems less important.

## 2. Results of attempts to transmit *P. inui* var. *cynomolgi* to healthy monkeys through the bites of infected mosquitoes.

### Experiment (22).

No difficulty was experienced in obtaining suitable gametocyte carriers for feeding experiments. Bred specimens of anopheline mosquitoes were found to feed readily on experimentally infected monkeys, and a high percentage of many batches of these insects became infected. Oöcysts and sporozoites of *P. inui* var. *cynomolgi* were observed in the following species—*A. annularis*, *A. maculatus*, *A. culicifacies*, *A. subpictus* and *A. splendidus*. On three occasions healthy specimens of *S. rhesus* were infected by the bites of infected specimens of *A. annularis* and once by *A. subpictus*.

**Result.**—No detectable change in the morphology of *P. inui* var. *cynomolgi* was observed in animals infected by insect transmission, as compared with that seen in animals infected by direct blood inoculation. The duration of the schizogony cycle also remained unchanged.

## 3. Summary and conclusions.

1. No changes in the morphology of the parasite, nor in the duration of its cycle of schizogony, could be detected when *P. inui* var. *cynomolgi* was

(a) passed from *S. rhesus* to *S. rhesus* through the bites of infected mosquitoes, and

(b) subsequently passed and sub-passaged to other specimens of *S. rhesus* by blood inoculation.

2. All attempts to transmit *P. knowlesi* through the bites of mosquitoes failed.

3. The mosquitoes used in these experiments proved much more susceptible to infection with *P. inui* var. *cynomolgi* than to infection with *P. knowlesi* under the same conditions. This is a further indication of the specific identity of these two parasites.

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\*Knowles (1932a) records that a total of over 1,000 mosquitoes (*A. stephensi*, *A. barbirostris*, *A. subpictus*, *Aedes aegypti* and *Culex fatigans*) were fed on *S. rhesus* and man infected with *P. knowlesi*. Of these 419 survived, and were dissected or sectioned. All were negative with the exception of one specimen of *A. stephensi* fed on *S. rhesus*. Oöcysts were found in this mosquito on 18th day after the infective feed. They 'resembled those of *P. malariae* as described in textbooks'. It was concluded that the transmitting vector in nature is probably some jungle-breeding species.

### E. DIFFERENCES IN THE PATHOGENICITY OF *P. KNOWLESI* AND *P. INUI* VAR. *CYNOMOLGI*.

Some workers on monkey malaria have suggested that pathogenicity may prove to be a factor of considerable importance in the differentiation of species of monkey *Plasmodium*. A comparison between the pathogenic effects produced by two species of *Plasmodium* may only be expected to be of value when each of the infections is observed in the same species of animal host. Animal hosts selected for such a comparison should be, as far as possible, from species which are free from plasmodial infections in nature, e.g., *S. rhesus* in northern India. The use of monkeys from endemic areas may lead to fallacies owing to the possible occurrence of varying degrees of acquired tolerance (Mulligan and Sinton, 1933; Sinton and Mulligan, 1933b).

#### 1. The pathogenic effects produced by *P. knowlesi* in different species of monkeys.

The pathogenicity of pure infections with *P. knowlesi* to various species of monkey has been discussed more fully in a previous paper (Sinton and Mulligan, 1933a), and it is only necessary here to give a brief summary of the previous findings.

##### Experiment (23).

In naturally infected specimens of *S. irus* no symptoms are observed, and the infection is detectable only by blood examination\*. Normal monkeys of the same species, inoculated experimentally with a pure strain of *P. knowlesi*, show mild infections and, beyond a slight rise of temperature daily in the early stages, no inconvenience appears to be caused to the animals. On the contrary, inoculation infections with *P. knowlesi* in *S. rhesus* are associated with severe, pernicious symptoms, and often with hæmoglobinuria. Unless adequate treatment be given early in the primary attack such infections prove rapidly fatal. Acute attacks with constitutional symptoms have also been observed in one specimen of *Pygathrix schistaceus*, and one of *S. sinicus*, but both of these animals recovered after treatment.

*Result.*†—In the specimens of *S. irus* studied by us, *P. knowlesi* produced mild effects in both natural and experimental infections. On the contrary infections with this parasite, experimentally produced in *S. rhesus*, *S. sinicus* and *Py. schistaceus*, are associated with acute attacks accompanied by constitutional symptoms. The infections in *S. rhesus* are particularly virulent.

#### 2. The pathogenic effects produced by *P. inui* var. *cynomolgi* in various species of monkeys.

##### Experiment (24).

No symptoms were observed in any of the monkeys (*S. irus*) which were found to be infected in nature.† Normal specimens of *S. irus*, inoculated with a pure strain

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\* There is good reason to believe that all the naturally infected specimens of *S. irus* observed by us were suffering from mixed infections with *P. knowlesi* and *P. inui* var. *cynomolgi*.

† Knowles and Das Gupta (1932) have recorded marked variations in the pathogenic effects of the two types of parasites observed by them. The mild infections observed in

of *P. inui* var. *cynomolgi*, did not appear to suffer any inconvenience, although in one case a moderately heavy parasitic infection was observed. After inoculation of this parasite into *S. rhesus*, parasitic infections of moderate severity were the rule, but in no case were the symptoms severe. Spontaneous recovery was invariable in all cases observed by us. In a single specimen of *S. sinicus* inoculated with this parasite, a very mild infection, unaccompanied by detectable symptoms, was observed.

**Result.**—The clinical symptoms produced by a pure infection with *P. inui* var. *cynomolgi* have remained remarkably constant and mild for each of the species of monkeys observed by us.

The mild effects produced in *S. rhesus* are in very marked contrast to the severe and often fatal infections seen in this species of monkey after inoculation with *P. knowlesi*. Similarly the symptoms observed in *S. sinicus* infected with the latter parasite are much more severe than those seen in this species of monkey after inoculation with *P. inui* var. *cynomolgi*.

### 3. Effects of cross-immunity.

Mulligan and Sinton (1933) have recorded the results of experiments on cross-immunity between these two parasites. No immunity was found, but this was to be expected if the parasites used were of two different strains, even of the same species. When, however, cross-immunity experiments were tried with *P. knowlesi* and *P. inui* var. *cynomolgi*, isolated from the same specimen of natural host, and therefore presumably of the same strain, no sign of tolerance was detected. This finding adds considerable support to the evidence that *P. knowlesi* and *P. inui* var. *cynomolgi* are two distinct entities.

### 4. Summary of experiments on pathogenicity.

The pathogenic effects produced when pure strains of either *P. knowlesi* or *P. inui* var. *cynomolgi* were inoculated into normal monkeys have remained remarkably uniform for each species of animal used in these experiments.

(a) *P. knowlesi* caused severe infections in both *S. rhesus* and *S. sinicus*. In the former species of monkey the acute attack was often accompanied by hæmoglobinuria, and proved fatal unless treatment was commenced in the early stages of the primary attack. In *S. irus* the infections produced by this parasite were of a very mild character, probably on account of the presence of either a natural or an acquired tolerance.

(b) Infections with *P. inui* var. *cynomolgi*, on the contrary, caused few or no symptoms in any of these three species of *Simulans*. In *S. rhesus* moderately heavy parasitic infections were encountered, but no severe or pernicious symptoms were observed. In all these animals spontaneous recovery was invariable.

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*S. irus* were, we believe, due chiefly to mixed infections in which *P. inui* var. *cynomolgi* was the predominant infection. Virulent infections with *P. knowlesi* were observed in *S. rhesus* and *Py. entellus*, while in *S. sinicus* milder infections, usually with spontaneous recovery, were seen. These findings are in close agreement with those recorded above.

(c) Chronic infection with *P. knowlesi* was not associated with any cross-immunity to *P. inui* var. *cynomolgi* and *vice versa*, even when the two species had been isolated from the same specimen of natural host.

### 5. Conclusions.

(a) Very striking differences have been observed between the pathogenic effects produced by these two parasites in various species of animal host. These add very considerable support to the evidence in favour of *P. knowlesi* and *P. inui* var. *cynomolgi* being two specifically distinct parasites, and not variations of a single species.

(b) The specific identity of each of these two Plasmodia is supported by the absence of any appreciable degree of cross-immunity between them.

### F. CONCLUSIONS.

Under the conditions of our experiments it has been found that :—

- (a) *P. knowlesi* and *P. inui* var. *cynomolgi* show very striking and characteristic morphological differences.
- (b) The duration of the schizogony cycle in *P. knowlesi* is 24 hours and that of *P. inui* var. *cynomolgi* 48 hours.
- (c) The alterations produced on the infested red cells are characteristic for each species of parasite.
- (d) These characters have remained constant, irrespective of the species of animal host through which either species of *Plasmodium* has been passaged. In the case of *P. inui* var. *cynomolgi*, passage through the mosquito has not been found to alter these diagnostic characteristics.
- (e) Marked variations in the immunity or tolerance of the animal host were not associated with any detectable changes in the specific characters of these two Plasmodia.
- (f) No forms intermediate between the 'rhesus' and the 'Cercopithecus' types of Knowles and Das Gupta (1932) were encountered under any of these conditions.
- (g) When observed in certain species of monkey, the pathogenic effects produced by these two parasites showed very considerable differences. This was particularly striking in *S. rhesus*.
- (h) One species of *Plasmodium* was not found to confer any cross-immunity to the other, even when both were isolated from the same specimen of natural host.

The evidence collected is so conclusive that there can be no reasonable doubt that *P. knowlesi* Sinton and Mulligan, 1932, and *P. inui* var. *cynomolgi* Mayer, 1907, are two distinct species and *not* variations of the same species of *Plasmodium*.

## II. DID EITHER *P. KNOWLESI* OR *P. INUI* VAR. *CYNOMOLGI* ORIGINATE FROM UNDETECTED LATENT INFECTIONS IN *S. RHEBUS*?

It was now established beyond any reasonable doubt that two definitely distinct species of *Plasmodium* were present in the infections in our animals. There appeared also to be little question that both species of *Plasmodium* had originated from naturally infected specimens of *S. irus*. However, the possibility that one or both of these parasites might have been derived from undetected latent infections in *S. rhesus* could not be overlooked. It appeared to us that this question was so important as to require more exhaustive investigation.

Knowles and Das Gupta (1932) and Knowles (1932a) had considered a similar explanation for the complete change of morphology which they had observed, when a natural malarial infection in *S. irus* was passaged by blood inoculation to other species and genera of monkey. They state that their findings were open to the obvious criticism that they 'were dealing with latent malaria in some of the 43 hosts used . . . . ., and that the inoculations awakened this latent malaria into activity'. They concluded that 'this, again, is certainly not the case. In all hosts used (with the exception of human volunteer V3) thin and thick blood films were examined and Bass cultures of the blood taken before inoculation; all these proved negative. We have already mentioned our previous want of success when searching batches of *M. rhesus* purchased locally for natural Plasmodia of their own. Also it is incredible that 43 consecutive Primate hosts belonging to 7 different species should all have had a latent and undetected malarial infection of their own. We have taken every precaution to use only "clean" hosts'.

We are in complete agreement with the reasons given by these authors for disregarding the possibility of latent infections as the source of the infections observed by them in inoculated animals. It was thought, however, that further evidence was needed to place such a conclusion beyond reasonable doubt. The results of our investigations, which are recorded below, strengthen the evidence against such an explanation of the origin of either parasite.

The occurrence of latent plasmodial infections in monkeys, exposed to infection in areas where monkey malaria is endemic, is probably much more common than is generally recognized (*vide* Appendix, Sinton and Mulligan, 1933b). It is usually only when such animals come under close observation in the course of laboratory experiments, that infections of this nature are liable to be detected. There are several instances on record where unsuspected latent malarial infections have been discovered accidentally, as the result of abnormal conditions in the host.

Sargent (1908) noted the sudden awakening of an infection with *P. kochi* following traumatism. Knowles (1919) discovered an infection with *P. semnopithecii* in *Pygathrix entellus*, after the animal had received an injection of human blood. Grigorieva (1929) reports the accidental discovery of malarial infections in two monkeys, and emphasises

the difficulty of detecting such infections. Berenberg-Gossler (1909) found an infection with *P. inui* in *S. irus*, following an injection of blood from *Brachyurus calvus*, an American monkey. Chimisso (1922) found a natural infection in a specimen of *S. rhesus* which had already been used for another experiment. Noguchi (1928) only discovered Plasmodia in a specimen of *S. irus*, after this animal had been splenectomized during the course of another experiment.

Some observers (Mayer, 1908; Gonder and Rodenwaldt, 1910) have noted that the blood of monkeys in which no parasites are detectable by microscopical examination, may prove infective to other monkeys. It has also been our experience that monkey blood may be infective to susceptible hosts, even when no parasites can be found in it. Such latent infections are, therefore, very easily overlooked, especially when their detection is dependent upon a few blood examinations only (*vide* Appendix I).

### 1. The possible occurrence of natural malarial infections in specimens of *S. rhesus* captured in northern India.

The occurrence of natural plasmodial infections in *S. rhesus* in India, has never been described, so far as we are aware. Considering that this species of monkey is commonly used for experimental work in India, it would be extraordinary if infections of this nature should have escaped detection in the past. *S. rhesus* is also a common laboratory animal all over the world, yet there appear to be only two reliable records of natural infections with Plasmodia in this species (*vide* Appendix, Sinton and Mulligan, 1933b). Chimisso (1922) reported a natural infection (identified as due to *P. semnopithecii* by Sinton and Mulligan, 1932) in a specimen of *S. rhesus* in Italy. This monkey was said to have been imported from India. Mathis and Leger (1911) record the occurrence of *P. inui* as a natural infection of *S. rhesus* in Tonkin. On the other hand, although thousands of specimens of *S. rhesus* must have been subjected to the most careful scrutiny in connection with the investigation of other diseases, we have been unable to find any other records of the occurrence of natural plasmodial infections. A few examples of such investigations are quoted below :—

Noguchi (1926-1928) examined about 50 specimens of *S. rhesus* while studying Oroya fever and verruga peruviana. Although the blood and tissues of these animals must have been subjected to minute examination in the search for *Bartonella bacilliformis*, yet no malarial infection is recorded. On the other hand a note was made when one out of 3 specimens of *S. irus* was found infected with *P. inui* (?).\*

Stokes, Bauer and Hudson (1928) examined about 90 specimens of *S. rhesus* in the course of their investigations of yellow fever. Most of these animals were carefully studied at autopsy, but no malarial infection was reported. Hudson (1928), in a separate paper, has described minutely the necropsy findings in 68 of these monkeys, and although the occurrence of tuberculosis and of other diseases is recorded, no mention is made of malaria. There is also nothing in the reported findings to suggest that malarial infection was present in any animal.

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\* This infection was latent and unsuspected. It was only discovered after the animal had been splenectomized (Noguchi, 1928).

Kucsynski and Hohenadel (1930), working on yellow fever, had occasion to examine 550 specimens of *S. rhesus*, but the occurrence of malarial infections among them is not mentioned.

Other records of the examination of large numbers of this species of monkey are not uncommon, but in none of those studied by us have we been able to find any mention of malaria, although other diseases have been reported.

Knowles and Das Gupta (1932) and Knowles (1932a) examined the bloods of many batches of monkeys, chiefly *S. rhesus*, during a number of years in Calcutta, in an unsuccessful attempt to find natural plasmodial infections. In their experiments with a natural malarial infection of *S. irus*, the bloods of all specimens of *S. rhesus* used for the passage of this infection were previously examined by both thin- and thick-film methods and by cultures. In no instance were they able to find a natural infection in this species of monkey.

We have had occasion to examine, carefully and repeatedly, the bloods of over 300 specimens of *S. rhesus* from northern India, without being able to detect the presence of Plasmodia. Particular attention has been paid to the detection of latent infections. In spite of the production of protein shock, of prolonged and repeated blood examinations, of isodiagnostic methods, of splenectomy and of a combination of several of these methods, no natural malarial infections have been discovered by us in *S. rhesus* (*vide* Appendix I).

## 2. Discussion.

There appears to be very considerable evidence to support the conclusions that natural malarial infections are very rare in *S. rhesus* in India, and that they are probably absent among this species of monkey in northern India. If such infections occur they must be of extreme rarity in the localities from which laboratory animals are usually collected.

If the strain of *P. knowlesi*, isolated by us in *S. rhesus* from naturally infected specimens of *S. irus*, were latent in the former species of monkey, it would be necessary to assume that by a remarkable coincidence only monkeys with latent infections were used for this purpose. It would also be a strange coincidence that all these animals should succumb to acute malarial attacks originating from their own latent infections, when numerous other monkeys inoculated by us from normal specimens of *S. irus*, as controls, should remain in perfect health. If such latent infections were present in *S. rhesus*, it is very curious that they were not re-awakened by the preliminary intravenous injection of human blood, which some of them received (*vide* Appendix I). The infections, which appeared in the same animals after the inoculation of blood from infected specimens of *S. irus*, were found to be easily stimulated by injections of human blood, made when these infections became latent at a later date. This evidence very strongly negatives the suggestion that any of the original specimens of *S. rhesus* used by us had undetected latent infections.

In practically all specimens of *S. rhesus* inoculated by us from naturally infected specimens of *S. irus*, parasites appeared in the peripheral blood within

a reasonable incubation period (usually about 4 or 5 days)\*. In the absence of treatment the infections proceeded to a fatal termination, with one exception. This would be a very unexpected result, if the infections in *S. rhesus* were merely the outcome of a stimulation of natural latent infections by protein shock. Our experience of the use of protein shock for the detection of latent infections has been that parasites appear in scanty numbers within about 24 hours, and that the resultant relapse is extremely mild and very transient. It would also be reasonable to expect that if the infections seen in *S. rhesus* originated from latent infections, some of these monkeys at least would have possessed sufficient tolerance, as the result of the chronic infection, to prevent the occurrence of such severe pathogenic effects.

Specimens of *S. irus* found infected in nature have been shown to harbour malarial parasites, the morphological and other characteristics of which are indistinguishable from those seen in specimens of *S. rhesus*, experimentally infected with *P. knowlesi*. It appears to us, therefore, that there can be little doubt but that the infections observed by us in the latter species of monkey resulted from parasites inoculated from naturally infected specimens of *S. irus*.

For similar reasons the strains of *P. inui* var. *cynomolgi* isolated by us in *S. rhesus* undoubtedly originated in naturally infected specimens of *S. irus*. One of these strains was detected in *S. rhesus* 11 days after this monkey had received an injection of blood from *S. irus*. The only detectable parasite in the blood of the latter animal at the time of inoculation was morphologically identical with that which subsequently appeared in *S. rhesus*. The other strain of *P. inui* var. *cynomolgi* was isolated as the result of the bites of mosquitoes fed on an animal suffering from a mixed infection. Parasites appeared in the blood of a healthy specimen of *S. rhesus*, 16 days after it had been bitten by infected mosquitoes.

### 3. Conclusions.

There can be little doubt, from the numerous observations recorded above, that our strains of both *P. knowlesi* and *P. inui* var. *cynomolgi* originated from natural infections in *S. irus*.

There is no evidence to show that either parasite was derived from an unsuspected or undetected latent infection in any of the specimens of *S. rhesus* used in our experiments.

### III. CAN THE PARASITIC BLOOD PICTURES OBSERVED IN NATURAL INFECTIONS IN *S. IRUS* BE REPRODUCED BY THE EXPERIMENTAL PRODUCTION OF MIXED INFECTIONS IN NORMAL MONKEYS ?

The presence of two different types of *Plasmodium* was observed in naturally infected specimens of *S. irus* and in monkeys infected from these. This suggested that mixed infections were possibly present.

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\* In only two cases the incubation period may have been as long as one month (Mulligan and Sinton, 1933).



As detailed earlier in this paper, two distinct species of *Plasmodium* have been isolated from the same naturally infected specimen of *S. irus*. It was thought that further proof of the specific identities and sources of these two parasites would be furnished, if it were possible to reproduce experimentally appearances comparable with those noted in naturally infected animals. It was, therefore, decided to study the appearances produced in normal monkeys by inoculation with both *P. knowlesi* and *P. inui* var. *cynomolgi*.

The experimental production of mixed infection with these two species of *Plasmodium* was effected by:—

(a) The inoculation of *P. knowlesi* into monkeys already infected with *P. inui* var. *cynomolgi* and *vice versa*.

(b) The simultaneous inoculation of pure infections of both species of parasite into normal monkeys.

(c) The inoculation of normal monkeys from other monkeys in which artificially produced mixed infections with these two parasites were present.

The results of these experiments are detailed below.

### 1. The inoculation of *P. knowlesi* into monkeys infected with *P. inui* var. *cynomolgi* and *vice versa*.

(a) *Established infections of P. knowlesi superinfected with P. inui var. cynomolgi.*

#### Experiment (25). Monkey No. 80 (*S. rhesus*).

This monkey, which was suffering from a chronic infection with *P. knowlesi*, was superinfected with a pure strain of *P. inui* var. *cynomolgi*. Blood films from this animal were examined daily and on the 5th day after superinfection both species of *Plasmodium* were detected. A moderately heavy infection with *P. inui* var. *cynomolgi* developed. This was similar in every respect to that usually observed in *S. rhesus* infected with a pure strain of this parasite, except that a small number of *P. knowlesi* forms could be detected by very careful examination. The latter parasite would have been overlooked had not the observer been very familiar with the morphological characteristics of *P. knowlesi*.

*Result.*—The blood picture seen in this monkey, after the forms of *P. inui* var. *cynomolgi* appeared, was comparable in all respects with the appearances observed in naturally infected specimens of *S. irus*.

#### Experiment (26). Monkey No. 121 (*S. irus*).

This animal, which was suffering from a mild infection with a pure strain of *P. knowlesi*, was superinfected with a pure strain of *P. inui* var. *cynomolgi*. Both species of *Plasmodium* were recognisable in the blood 9 days after superinfection. Subsequently the blood picture was identical with those seen in naturally infected specimens of *S. irus*. A chronic malarial infection was observed in this animal for several months. At some examinations the predominant infection was one of *P. knowlesi*, while at others *P. inui* var. *cynomolgi* was more conspicuous. Further observations were made on this monkey after splenectomy [*vide* experiment (19)].

*Result.*—A parasitic infection was produced which appeared to be identical with the 'Cercopithecus type', described by Knowles and Das Gupta (1932) and seen by us in natural infections in *S. irus*.

(b) *Established infection of P. inui var. cynomolgi superinfected with P. knowlesi.*

**Experiment (27). Monkey No. 93 (*S. rhesus*).**

This animal was suffering from a chronic infection with *P. inui* var. *cynomolgi* and was superinfected with a pure strain of *P. knowlesi*. The blood of the animal was carefully examined, and 14 days after superinfection both species of *Plasmodium* were detected. *P. knowlesi* very rapidly became the predominant infection. No treatment was given and the latter infection caused the death of the animal within 3 days of its first recognition in the blood.

**Result.**—The blood picture on the day when *P. knowlesi* was first detected closely resembled that seen in natural infections in *S. irus*. *P. knowlesi*, however, quickly predominated.

**2. The simultaneous inoculation of *P. knowlesi* and *P. inui* var. *cynomolgi*.**

**Experiment (28). Monkey No. 116 (*S. rhesus*).**

This normal monkey received, on the same day, an inoculation with pure strains of both *P. knowlesi* and *P. inui* var. *cynomolgi*. Very scanty parasites were detected in the blood 2 days later. During the early stages of the infection, very careful scrutiny of the blood revealed the presence of both species of *Plasmodium*. *P. knowlesi* very quickly became the predominant species, hæmoglobinuria developed, and death quickly ensued. Blood films taken just before death showed an extremely heavy infection with *P. knowlesi*, but a few forms of *P. inui* var. *cynomolgi* could also be found on careful examination. It is very unlikely that the latter forms would have been recognized, unless a special search had been made for them by an expert observer.

**Result.**—This was very like the effect produced when *S. rhesus* is inoculated with a natural mixed infection, and the animal died in the absence of treatment.

**Experiment (29). Monkey No. 161 (*S. irus*).**

This normal monkey received a simultaneous inoculation with pure strains of both species of *Plasmodium*. Parasites appeared after an incubation period of 5 days. At first only *P. knowlesi* was detected, but within 2 days both species of *Plasmodium* were recognizable in the blood. *P. knowlesi* was the predominant parasite for the first 17 days of the infection, after which *P. inui* var. *cynomolgi* became the more conspicuous species. The infection was a mild one and parasites were never abundant at any stage. A low-grade infection was subsequently observed for 6 weeks. At first the predominant parasite found at some examinations was *P. knowlesi*, but *P. inui* var. *cynomolgi* later became the only detectable one.

**Result.**—The blood picture in this monkey appeared to be identical with that observed in some of our naturally infected specimens of *S. irus*, and in other animals of this species infected from them.

**3. The inoculation of normal monkeys from others with experimentally produced mixed infections.**

**Experiment (30). Monkey No. 112 (*S. rhesus*).**

This normal monkey was inoculated from *S. rhesus* (No. 80) [vide experiment (25)], in which a mixed infection had previously been produced experimentally. At the time

of inoculation both species of parasite were detectable in the blood of the latter monkey. Parasites appeared in the inoculated animal after an incubation period of 8 days. In the early stages of the infection it was possible by careful examination to detect both species of *Plasmodium*. No treatment was given, and, within 6 days after the first appearance of parasites, the monkey died with an acute attack of malaria.

**Result.**—Blood films taken just before death showed an extremely heavy infection with *P. knowlesi*. No forms of *P. inui* var. *cynomolgi* could be distinguished with certainty. This blood picture was identical with that seen in *S. rhesus* inoculated from a naturally infected specimen of *S. irus*.

#### Experiment (31). Monkey No. 94 (*S. rhesus*).

This normal monkey was also inoculated with the experimentally produced mixed infection of *S. rhesus* (No. 80) (*vide supra*). At the time of inoculation the latter monkey showed both species of *Plasmodium* in its peripheral blood. Parasites appeared in the inoculated animal after an incubation period of 5 days. In the early stages of the infection both species of parasite were recognizable on careful examination *P. knowlesi* became predominant so rapidly that treatment was necessary to save the animal's life. As a result of the treatment *P. inui* var. *cynomolgi* disappeared from the peripheral blood, while *P. knowlesi* persisted for 4 days. A relapse occurred 6 days after the cessation of treatment in which *P. knowlesi* was the only parasite detected. Further treatment was needed during this relapse. Subsequently two other relapses occurred and in each the latter parasite was the only species seen. The monkey died in the last relapse which was untreated\*. From the time when treatment commenced until death occurred, *P. knowlesi* was the only parasite detected.

**Result.**—The absence of *P. inui* var. *cynomolgi* from the peripheral blood after treatment commenced is very similar to observations made on specimens of *S. rhesus* inoculated from natural mixed infections in *S. irus*.

#### Experiment (32). Monkey No. 154 (*S. rhesus*).

This normal animal was inoculated from *S. irus* (No. 121), which had previously been experimentally infected with both species of *Plasmodium* [*vide* experiments (3) and (26)]. At the time of inoculation the only detectable parasite in the peripheral blood of the donor animal was *P. inui* var. *cynomolgi*, i.e., a blood picture resembling that seen in natural infections in *S. irus*. Parasites appeared after an incubation period of 5 days. In the early stages the only detectable parasite was *P. knowlesi*. This infection developed rapidly, and treatment was necessary to save the life of the animal. A chronic infection with this parasite developed, and, although daily blood examinations were made, *P. inui* var. *cynomolgi* was not detected until 6 weeks after the date of inoculation. At the end of this period the latter parasite soon became the predominant species. Recovery from the latter infection was spontaneous, and subsequently both species of *Plasmodium* were recognizable at times. *P. knowlesi* was, however, usually the predominant form. Splenectomy was subsequently performed on this animal [experiment (20)].

**Result.**—An acute attack due to *P. knowlesi* occurred. Although an infection with *P. inui* var. *cynomolgi* appeared at the end of 6 weeks, the former parasite predominated in the later stages of the chronic infection.

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\*The death of this monkey during a relapse was the first we have encountered in attacks other than the primary one. It is possible that some of the other monkeys observed by us might have died under similar conditions if treatment had been withheld.

#### 4. Discussion on the significance of mixed infections produced experimentally.

The results of the experiments recorded above show that it is possible to produce mixed plasmodial infections in monkeys. It is also possible in such infections to distinguish each species of parasite by its morphological characteristics. The appearances produced in such experimental mixed infections are, at certain stages, comparable in every respect to those seen in naturally infected specimens of *S. irus*, and in other monkeys of the same species inoculated from the latter. This finding holds good, irrespective of whether the mixed infections produced experimentally are observed in *S. rhesus* or *S. irus*. The species of *Plasmodium*, which predominates in the peripheral blood in such mixed infections, depends largely upon the degree of tolerance present at the time in the animal inoculated. These findings add considerable support to the view that the natural infections observed by us in *S. irus* were mixed ones, and that both *P. knowlesi* and *P. inui* var. *cynomolgi* were present in such infections.

When the blood from monkeys with experimental mixed infections was inoculated into normal specimens of *S. rhesus*, the resultant infection was predominantly one of *P. knowlesi*. These animals rapidly succumbed to the infection, if treatment was not given. This result is very similar to that observed when the same species of monkey is inoculated from naturally infected specimens of *S. irus*.

Both *P. knowlesi* and *P. inui* var. *cynomolgi* retain their characteristic morphology and duration of schizogony cycle in such artificially produced mixed infections. This fact appears to us to furnish the clearest proof that individual or specific idiosyncrasies on the part of the inoculated animals have no rôle in determining the special characteristics of either species of *Plasmodium*. If such idiosyncrasies on the part of the animal host were concerned in determining the morphology, etc., of these parasites, the appearances observed in any particular animal should be of one type only. On the contrary, both types of *Plasmodium* can be distinguished with ease in the same monkey host, sometimes concurrently and sometimes consecutively.

In many animals it has been found possible to detect the presence of both species of *Plasmodium* in the very early stages of infection. In normal specimens of *S. rhesus*, *P. knowlesi* becomes the predominant species so quickly that *P. inui* var. *cynomolgi* is very liable to be overlooked. The latter species may be detected in many cases, if a special search be made by an observer who is thoroughly familiar with the differential characteristics of the two parasites. The difficulty of recognizing the presence of mixed plasmodial infections in monkeys, when only scanty parasites are present, will be appreciated by anyone who has experienced the difficulty of detecting such infections in man. It is not an easy matter to recognize the presence of mixed infections in human malaria, in the absence of such outstandingly characteristic forms as the more typical trophozoites of *P. vivax* or the crescents of *P. falciparum*. It is, therefore, not surprising that the presence of mixed infections,

due to parasites so little known as the *Plasmodia* of monkeys, should have escaped detection in the past.

Another fact came to light as the result of the above experiments. It is not necessarily the predominant parasite in mixed infection which appears first, or most abundantly, in the animal to which the mixed infection has been passaged. This is not difficult to understand when one realizes that the blood of one monkey may be infective to another, even when no parasites can be detected at repeated blood examinations. The species of parasite which predominates appears to be determined by the degree of immunity or tolerance, present at the moment, in the recipient host. The results of these experiments bear a striking resemblance to those recorded by Antic (1925) in his investigation of mixed infections in human malaria.

Some of our experiments described above have suggested that the treatment given to control the acute attacks may, to some extent, determine the parasite which predominates at a later stage of infection. The treatment given in acute attacks due to *P. knowlesi* appears to be sufficient to eradicate in some cases, or at least suppress for long periods in others, the concomitant infection with *P. inui* var. *cynomolgi*. The significance of this observation, and the results of experiments made to test this hypothesis, are considered later in this paper.

### 5. Conclusions.

The results recorded above show that under the conditions of our experiments:—

(a) Mixed infections can be reproduced experimentally by the inoculation of pure infections of *P. knowlesi* and *P. inui* var. *cynomolgi* into the same monkey.

(b) The parasitic picture observed in *S. irus* under these conditions is apparently identical with that seen in natural infections in this species of monkey.

(c) Similar appearances are seen in specimens of *S. rhesus*, in which some degree of tolerance has been established to *P. knowlesi*, before superinfection with *P. inui* var. *cynomolgi* is carried out.

(d) In such mixed infections, both species of *Plasmodium* are detectable in the blood of the recipient animal at some stage of the infection.

(e) Each species retains its own morphological characteristics and duration of schizogony cycle in such experimental mixed infections.

(f) The blood picture present at any one time depends upon the degree of tolerance or immunity present to each species of *Plasmodium* at that time.

(g) Passage by blood inoculation of infection from experimental mixed infections, produced the same blood picture and pathogenic effects as did similar inoculations from natural mixed infections, if the same type of animal were used.

#### IV. WHY IS *P. KNOWLESI* THE PREDOMINANT, AND OFTEN THE ONLY, PARASITE DETECTED IN *S. RHESUS* MONKEYS INOCULATED FROM NATURALLY INFECTED SPECIMENS OF *S. IRUS*?

The predominant or more conspicuous infection in naturally infected specimens of *S. irus* is usually *P. inui* var. *cynomolgi*. On the other hand, when specimens of *S. rhesus* are inoculated from the former animals, the predominant, and possibly the only detectable, infection is almost invariably *P. knowlesi*. This reversal of the predominant species of *Plasmodium* is the usual result seen with the animals of these two species used in our work. It was apparently due to this phenomenon that Knowles and Das Gupta (1932) were convinced that they were dealing with a single species of *Plasmodium*, which changed its morphology in different species of monkey.

It is not a new observation that the predominant parasite in a known mixed infection, or even in an apparently pure one, may not be the species which predominates in the sub-inoculated host. In the early work on the malarial therapy of mental diseases, several instances are on record in which the inoculation of an apparently pure natural infection with *P. vivax* resulted in a severe infection with *P. falciparum*, sometimes followed by death\*. Such occurrences show a striking resemblance to some of our observations with experimental monkey malaria.

##### 1. Consideration of the possible causes of the predominance of *P. knowlesi* in *S. rhesus*.

Several explanations suggest themselves to account for the predominance of *P. knowlesi* in specimens of *S. rhesus* inoculated from natural mixed infections in *S. irus*.

##### (a) The extreme susceptibility of *S. rhesus* to infection with *P. knowlesi*.

The very marked differences between the pathogenic effects produced by *P. inui* var. *cynomolgi* and *P. knowlesi* in *S. rhesus* have been referred to earlier in this paper. Infections with *P. knowlesi* develop with great rapidity after inoculation into normal specimens of this monkey. This is due to its 24-hour cycle of schizogony, and possibly also to the absence of any tolerance, either natural or acquired, in *S. rhesus*. Within a few days of the appearance of parasites, an extremely heavy parasitic infection is present. When, however, specimens of *S. rhesus* have been immunized against this *Plasmodium* by super-infection, the pathogenic effects are slight, and the rapidity of development of

\*These observations were thought at one time to give considerable support to the unicity theory of the human Plasmodia. This apparent pleomorphism shows a very striking resemblance to the results recorded by Knowles and Das Gupta (1932) with regard to the malarial parasites of *S. irus*.

the infection very much less (Mulligan and Sinton, 1933). This result is very similar to that observed when an apparently normal specimen of *S. irus* is inoculated with the same parasite. The latter species of monkey, as seen in India, appears to have a considerable degree of tolerance to infection with *P. knowlesi*. This tolerance may be a natural characteristic of *S. irus*, but is more probably the result of previous infection in earlier life, i.e., a condition similar to that seen in experimentally immunized specimens of *S. rhesus*.

In both species of monkey, infections with *P. inui* var. *cynomolgi* are of a much milder character and develop more slowly.

Under these conditions, when a normal specimen of *S. rhesus* is inoculated with a mixed infection from a naturally infected *S. irus*, it is only to be expected that *P. knowlesi* will rapidly become the predominant infection in the recipient animal.

Several observations in connection with inoculation infections of human malaria are very similar to those mentioned above. When blood from a patient showing numerous forms of *P. vivax* in the peripheral blood, but also with an unsuspected infection of *P. falciparum*, is injected into an uninfected person, the latter parasite rapidly predominates in the acute stages of the resultant infection. This experience is identical with that found when an unsuspected mixed infection in *S. irus* is transmitted to *S. rhesus*. The peripheral blood of the donor animal shows an infection, which may seem to be a pure one of *P. inui* var. *cynomolgi*, while in the inoculated animal the more virulent parasite, *P. knowlesi*, predominates.

As noted above, a similar result was obtained when both species of parasite could be detected in the blood of the donor animal. This result is independent of whether the mixed infection was an experimental or a natural one. Antic (1925) found that, when he gave a simultaneous intravenous injection of both *P. vivax* and *P. falciparum* into a human patient, he was able to detect the former parasite in thick films on 2nd day. *P. falciparum* appeared on 8th day, at which time *P. vivax* disappeared. The patient died in coma on 13th day. This result shows a remarkable resemblance to the findings in some of our experiments [*vide* experiments (28) and (30)].

The disappearance, or marked diminution in the prevalence, of one species of parasite from the peripheral blood, in the presence of an acute infection due to a different species, is a well-recognized phenomenon in human malaria. Thus many unsuspected mixed infections show *P. falciparum* during the acute attack, and *P. vivax* at a later date. This phenomenon is discussed by Sinton and Mulligan (1933b). The observations, recorded in experiments (25) to (32), are examples of such occurrences with mixed infections of *P. knowlesi* and *P. inui* var. *cynomolgi*.

It is evident from the observations of Antic (1925) that, under similar conditions in human malaria, both species of parasite may be detectable in the peripheral blood very shortly after inoculation, if a special search be made. This may also be the case in monkey malaria [*vide* experiments (28) to (31)].

In the later stages of an acute infection in human malaria, it has been found that it is usually only possible to detect the presence of one species of parasite at any one time. A similar condition occurs in monkey malaria.

When two species of parasite are detectable in human malaria at the same time, it is more commonly the asexual forms of one and the sexual forms of the other. This phenomenon also occurs in mixed infections in simian malaria. Even when the gametocytes of *P. inui* var. *cynomolgi* are only apparent in comparatively scanty numbers in the presence of a predominant infection of *P. knowlesi*, it is still possible to infect mosquitoes with the former parasite (*vide* Appendix II).

(b) *The effects of treatment in causing predominance of infections of P. knowlesi in S. rhesus.*

In normal specimens of *S. rhesus*, an infection with *P. knowlesi* is so rapidly progressive and so extremely severe, that it has always been necessary in our work to commence treatment early, if the life of the animal is to be saved. The amount of treatment given to control the acute attack and subsequent relapses may be sufficient in many cases to eradicate or suppress any scanty or undeveloped infection with *P. inui* var. *cynomolgi*, which may be present. If treatment be withheld, death results from the infection with *P. knowlesi*. This probably occurs before the more slowly developing infection with *P. inui* var. *cynomolgi* has had time to be recognized, if previously unsuspected. The occurrence of death before the more scanty infection has become conspicuous in the peripheral blood, is what often occurs when mixed infections of *P. vivax* and *P. falciparum* are inoculated into susceptible human subjects.

The following experiments were carried out in an attempt to determine the relationship of treatment to the predominance of *P. knowlesi* in the peripheral blood of *S. rhesus*.

Experiment (33). Monkeys Nos. 94 and 137 (*S. rhesus*).

Two normal specimens of *S. rhesus* were selected. One of these, No. 94 [*vide* experiment (31)], received an inoculation from *S. rhesus* (No. 80) suffering from a mixed infection of *P. knowlesi* and *P. inui* var. *cynomolgi*. The other monkey, No. 137, was injected with *P. inui* var. *cynomolgi* only. The same strain of the latter parasite was used in both monkeys. Parasites appeared in both animals on the same day.

The first animal (No. 94) developed an acute infection with *P. knowlesi*, and treatment was given to control the attack. This was also necessary during two subsequent relapses due to the same parasite. The animal died during a third relapse for which no treatment had been given. Although a few forms of *P. inui* var. *cynomolgi* were detected on the first day when parasites were seen, they could not be found again at any time after the commencement of treatment. This was in spite of the fact that a very careful search was made daily up till the time of death, i.e., 40 days after the inoculation was made.

The control monkey (No. 137) received the same treatment, in the same doses and at the same times, as that given to Monkey No. 94. The blood of the former animal was subjected to the same careful scrutiny daily, and records were kept of the



results of the examinations Parasites could be detected in the blood of this monkey for the first 3 days, and subsequently from 16th to 20th days. On no occasion were more than a few scanty parasites seen. The number of parasites was so very few that they would easily have been overlooked in the presence of a very heavy infection with *P. knowlesi*. Examinations of the blood of this monkey were continued for over 3 months, but the presence of parasites was not subsequently observed, although no treatment had been given after 20th day.

**Result.**—In both these monkeys the presence of *P. inui* var. *cynomolgi* could be detected by careful examination in the very early stages of infection. After the commencement of treatment this species of *Plasmodium* was only seen in very scanty numbers, and for very transient periods, in the blood of the control monkey, No. 137. In Monkey No. 94, the infection with *P. inui* var. *cynomolgi* was either (a) suppressed by the acute infection with *P. knowlesi*, (b) eliminated by the treatment given to control the latter infection, or (c) was so scanty that it baffled detection in the presence of the heavier infection with *P. knowlesi*.

This experiment shows clearly that, in some instances at least, the treatment given to control infections of *P. knowlesi* in *S. rhesus* is sufficient to prevent the detectable presence of *P. inui* var. *cynomolgi*, if the two parasites be inoculated at the same time. This seems especially the case if, as in natural infections, the latter parasite be present in only scanty numbers in the blood inoculated. It also shows that infection with *P. inui* var. *cynomolgi* may be suppressed, and probably eradicated completely in some cases, by this amount of treatment. The amount of treatment given to Monkey No. 94 was intended to be the minimum which would prevent the death of the animal. That less than this minimum had been given is shown by the fact that the animal died during the 3rd relapse.

That the treatment needed to control infections of *P. knowlesi* in *S. rhesus* is not always sufficient to prevent the appearance of *P. inui* var. *cynomolgi* at a later date is shown by the results of the experiments given below. This seems more especially the case after the strains of parasite, used in mixed infections, have had some opportunity of becoming 'acclimatized' to *S. rhesus* by frequent passage.

#### Experiment (34). Monkey No. 154 (*S. rhesus*).

This animal [vide experiment (32)] received an inoculation from *S. irus* (No. 121), known to have an experimental mixed infection. At the time of inoculation *P. inui* var. *cynomolgi* was the only parasite detectable in the peripheral blood of the latter animal. After an incubation period of 5 days, an infection with *P. knowlesi* developed in the experimental animal. Minimal treatment was given to control the infection.

**Result.**—After treatment a chronic infection developed, in which *P. knowlesi* was the only detectable parasite in the early stages. Six weeks after inoculation, a typical infection with *P. inui* var. *cynomolgi* developed.

#### Experiment (35). Monkey No. 138 (*S. rhesus*).

This normal animal was inoculated with an infection of *P. inui* var. *cynomolgi* only. Parasites appeared on 5th day and an attack of moderate severity developed.

Treatment was given from 8th to 11th days, and all parasites had disappeared from the peripheral blood on 12th day. Although no parasites were seen, quinine was again given from 20th to 23rd days. The blood remained free from parasites until 6 weeks after the original inoculation, when a typical attack with *P. inui* var. *cynomolgi* developed. This attack underwent spontaneous recovery.

*Result.*—The short courses of treatment given were not sufficient to eradicate completely a simple infection with *P. inui* var. *cynomolgi*. This infection reappeared at a later date.

These two experiments show that short courses of treatment may be sufficient to prevent the development of *P. inui* var. *cynomolgi* in appreciable numbers in *S. rhesus* for as long as 6 weeks after inoculation. The infection may, however, relapse at the end of this time in some cases. Unfortunately we have no definite record of a parallel instance in the case of *S. rhesus* inoculated from a natural infection in *S. irus*. In some of our earlier work, however, before the accurate recognition of the two distinct species of *Plasmodium*, it was noted that in some chronic infections in *S. rhesus* derived from *S. irus*, certain of the infested red cells showed marked stippling. At the time the significance of this observation was not fully understood, but in the light of more recent work, there is little doubt that this stippling was associated with a relapse of *P. inui* var. *cynomolgi* in these mixed infections.

### (c) Discussion.

It is very probable that both the factors discussed above are involved, to varying extents, in causing the predominance of *P. knowlesi* in both acute and chronic mixed infections in *S. rhesus*.

In the acute stages, the greater virulence and the more rapid multiplication of this parasite in normal specimens of *S. rhesus* quickly makes it the predominant parasite. The more benign character and less rapid multiplication of *P. inui* var. *cynomolgi* makes it less conspicuous, and more quickly suppressed, in the presence of the acute infection with *P. knowlesi*. It can usually be detected, however, in the very early stages if a careful and expert examination be made. If treatment be withheld, the animal dies before the former parasite has an opportunity of becoming predominant. These findings are almost identical with those reported by Antic (1925), when *P. falciparum* and *P. vivax* are injected simultaneously into human patients.

The predominance of *P. knowlesi* in the peripheral blood is aided by the effects of treatment in the acute attack. The more abundant parasite persists longer in the presence of quinine treatment, which aids the suppression of the scantier infection with *P. inui* var. *cynomolgi*. This is parallel with the fact that in acute mixed infections of *P. falciparum* and *P. vivax*, the former parasite persists longer in the peripheral blood than the latter.

These two factors, (a) suppression of *P. inui* var. *cynomolgi* by the acute infection with *P. knowlesi* and (b) rapid elimination of the scantier forms of the former parasite by quinine treatment, would account for the predominance,

and apparently pure infections, of *P. knowlesi* recorded in acute infections in *S. rhesus*.

In chronic infections the former factor has less influence, while the amount of treatment previously given appears to be the main reason for the predominance of *P. knowlesi* at this stage. The experimental evidence suggests that the treatment given to control the acute attacks due to *P. knowlesi* will completely eliminate the infection with *P. inui* var. *cynomolgi* in some cases. In other instances, this treatment causes the latter infection to remain latent over long periods, just as has been observed with *P. vivax* in mixed infections of this parasite and *P. falciparum*. The relapses of *P. inui* var. *cynomolgi* are usually of such a benign character that they would remain undetected in most cases if daily blood examinations were not made. No clinical symptoms are detectable to suggest that such a relapse is present, so there is no indication, as in human malaria, of the occurrence of such a condition.

The more obvious presence of the 'Cercopithecus type' of parasite (*P. inui* var. *cynomolgi*) in infections in *S. irus* is largely due to the effects of immunity or tolerance in this host. In such animals, a considerable degree of resistance to the multiplication of *P. knowlesi* and tolerance to its pathogenic effects is present. The result is that infections with this parasite usually remain at a very low level at all times, at least in the type of animal available for our experiments. Just as Schüffner's dots make *P. vivax* parasites more conspicuous than *P. falciparum* in human malaria infections, so does a similar cellular change in the case of *P. inui* var. *cynomolgi*. In some instances, the findings suggest that in monkey malaria this resistance to parasitic invasion may be more active against *P. knowlesi* than against *P. inui* var. *cynomolgi* in these tolerant animals. This point requires further investigation.

In the presence of the more conspicuous infection with *P. inui* var. *cynomolgi* in *S. irus*, a scantier infection with *P. knowlesi* may easily be overlooked. This is especially the case if the observer be unfamiliar with the differential characteristics of the two species of parasite, and a special search is not being made for mixed infections. These factors seem to have been largely responsible for the complete change in morphology of the parasite, recorded when infections in *S. irus* are transmitted to *S. rhesus*.

## 2. Conclusions.

Under the conditions of our experiments, it was concluded that the predominance of *P. knowlesi*, the 'rhesus type' of parasite, in mixed infections of normal specimens of *S. rhesus* was determined by

- (a) the greater virulence and more active multiplication of this species of *Plasmodium*, in the absence of any tolerance in the animal host, and
- (b) the greater and more rapid action of treatment on *P. inui* var. *cynomolgi* in such infections.

The apparent predominance of *P. inui* var. *cynomolgi*, the 'Cercopithecus type' of parasite, in mixed infections in *S. irus* is probably due to

(c) the more conspicuous appearance of this parasite,

(d) the resistance of the animal host to any very active multiplication of *P. knowlesi*, and

(e) failure in the past to recognize the presence of scanty forms of the latter parasite in the peripheral blood, at the same time as *P. inui* var. *cynomolgi*.

## V. SUMMARY AND CONCLUSIONS.

Under the conditions of our experiments, the following results were obtained, and from these certain conclusions have been drawn.

(1) Two distinct species of *Plasmodium* have been isolated from specimens of *S. irus* found infected in nature. One of these parasites has been identified as *P. inui* var. *cynomolgi* Mayer, 1907, and the other as *P. knowlesi* Sinton and Mulligan, 1932. The former parasite corresponds to the 'rhesus type', and the latter to the 'Cercopithecus type' of *Plasmodium* described by Knowles and Das Gupta (1932).

(2) The morphological characters and the duration of the schizogony cycle of each of these two species of *Plasmodium* have remained constant :—

(a) when passaged and sub-passaged through different species of lower monkey,\*

(b) under conditions of extreme variation in the immunity or tolerance of the animal host, and,

(c) in the case of *P. inui* var. *cynomolgi*, after passage through the mosquito host.

(3) Marked differences were observed between the pathogenic effects produced by these two parasites in different hosts of the genus *Silenus*. These effects were remarkably constant for each species of *Plasmodium*.

(4) Both species of *Plasmodium* originated from the same species of natural host, *S. irus*.

(5) Even when isolated from the same specimen of *S. irus*, neither of these two parasites has shown any cross-immunity against infection with the other.

(6) Striking changes in the morphology, pathogenicity, etc., have been reported when malarial parasites derived from *S. irus* are passaged to other Primate hosts of different species and genera. It is concluded that these apparent changes are attributable to the presence of undetected mixed infections in *S. irus*.

(7) The marked predominance of *P. knowlesi* in *S. rhesus*, as compared with *S. irus*, is accounted for by the differences in the pathogenicity of the two parasites, and in the relative degrees of tolerance of the animal hosts.

(8) The occurrence of natural malarial infections in specimens of *S. rhesus* indigenous in northern India is unknown. If such infections occur, they must be of extreme rarity.

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## APPENDIX I.

## METHODS OF DETECTING LATENT PLASMODIAL INFECTIONS IN MONKEYS.

The interpretation of the work recorded in this paper depends, to a large extent, on whether the monkeys used for passage and other experiments, were free from latent malarial infections or not. Particular attention was, therefore, given to the detection of latent infections in all monkeys before using them in these investigations. It is proposed to describe these methods in some detail, in the hope that the experience gained in these experiments may be of service to other workers in similar investigations.

## (a) Blood examinations.

In several cases of monkey malaria observed by us for upwards of a year, it was noted that parasites may be apparent in the blood, only at very irregular intervals. No parasites may be detectable for long periods, in spite of many careful and prolonged examinations by the thick-film method\*, but may be seen at later examinations. Occasional and irregular examinations of smears of the peripheral blood cannot, therefore, be relied upon either to detect, or to exclude the possibility of, latent malaria in monkeys. If, however, the blood be examined daily over long periods, the chances of detecting an infection are very considerably increased. It is probable that the vast majority of latent malarial infections in monkeys would be detected, if careful examinations by the thick-film method were carried out at regular short intervals, at least weekly for several months. Such a method is a very tedious and protracted one (Stephens *et al.*, 1917-1921; Acton, 1921; Sinton, 1926a).

## (b) Cultural methods.

Sinton (1922) advocated the use of cultural methods as an aid to the diagnosis of human malaria, in cases where examinations of the blood failed to reveal the presence of parasites. These methods are also recommended by

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\* In the records of our work in this paper, 'blood examination' means examination by the thick-film method, unless where otherwise stated. In most instances both thin and thick films were studied. The thick-film method used is that described by Sinton and Banerjee (1925).

Knowles and Senior White (1930) for the detection of very scanty parasites in the peripheral blood. Knowles and Das Gupta (1932) used this method to exclude the possibility of latent infections in all the secondary hosts used by them, prior to inoculating these animals with infected blood.

While such methods are undoubtedly a very valuable aid in the diagnosis of malarial infections, a negative result cannot be taken to exclude the possibility of malarial infection. It is well known that sometimes, for unknown reasons, parasites may not develop in culture. The failure of inoculation experiments into susceptible hosts on some occasions shows that, even when an infection is present, the number of parasites in the peripheral blood may be too scanty to cause infection (*vide* Leger and Bouilliez, 1913). It seems probable that under such conditions cultural methods would also fail.

Cultural methods were not employed in our work, because it was considered that other methods, more easily carried out in the case of monkeys, would give more reliable results (*vide infra*).

Knowles and Das Gupta (1932) have advocated the use of cultural methods for the detection of mixed infections in monkey malaria. In our present state of knowledge, these methods do not seem to us to be reliable in all cases of monkey malaria, for the following reasons :—

(a) Parasites of the predominant species may be so numerous that the presence of an occasional parasite of another species in the cultures may be overlooked, because

(i) its numbers are so scanty, and

(ii) in the later stages of cultures, there is frequently a considerable degree of change in the morphology of some of the parasites, as compared with those seen in the blood stream. It may, therefore, be difficult to decide whether these altered parasites are the result of some such change, or of the presence of a mixed infection.

(b) The well-recognized characteristics of the human malaria parasites make mixed infections with these species more easily detectable in cultures. Unfortunately the differential characters of the simian *Plasmodia* have not been so clearly defined, so a peculiar parasite may be overlooked or attributed to the cultural changes mentioned above.

For these reasons cultural methods have not been employed by us in the detection of mixed infections.

### (c) Intravenous injection of foreign protein.

The use of protein shock for the detection of latent infections in human malaria has been advocated by several workers in the past. Parasitic relapses may follow in a number of cases. On the other hand, many infections are not stimulated to such a degree that the parasites can be detected by microscopical examination of the peripheral blood within a short period after the administration of the shock (Sinton, 1926b).

Berenberg-Gossler (1909) noted that the protein shock, produced by the injection of blood from a monkey of a different genus into an apparently normal animal, re-awakened an unsuspected latent infection in the latter monkey. Gonder and Rodenwaldt (1910) were able to bring about a reappearance of parasites in the blood of monkeys following injections of horse serum. Mulligan and Sinton (1933) record that the intravenous injection of foreign protein, such as 2 or 3 c.c. of human blood into monkeys suffering from latent malarial infections, often caused the reappearance of parasites in the peripheral blood. The latter workers point out that a negative result cannot be accepted as proof that the animal is completely free from infection. Recently we have obtained encouraging results by the intravenous injection of 2 c.c. of normal horse serum.

Methods of protein shock appear to facilitate the detection of latent infections in many cases of monkey malaria. When combined with the 'isodiagnosis method' described below, it is probable that extremely few, if any, latent infections escape detection.

#### (d) Isodiagnosis.

It is well known that the blood of birds with latent malarial infections may be infective to healthy birds, even when it has been impossible to detect the presence of parasites after prolonged examination. In the opinion of many workers this method of 'isodiagnosis' is an extremely delicate test for the presence of latent malarial infections in birds. It seemed probable, therefore, that this method might prove to be equally valuable for the detection of latent infections in monkeys.

In the protocols of experiments recorded by Leger and Bouilliez (1913), reference is made to two monkeys (Nos. 46 and 94) whose blood proved infective to healthy animals, although no parasites were detectable at the time of inoculation. The same workers also report that the blood of animals, known to have latent infections, may fail to cause infection when injected into healthy animals. On the other hand blood taken at a later date and inoculated into the same animals proved to be infective (*vide* Monkeys Nos. 609 and 856).

It has been our experience that extremely small doses of blood, in which very scanty parasites are detectable, may be infective to healthy monkeys. Even when parasites can only be detected with difficulty in the thick film, doses of 0.1 c.c. of such blood from an infected animal (*P. knowlesi*) have very seldom been known to fail to cause an infection\*.

We have also succeeded in infecting healthy specimens of *S. rhesus* with *P. knowlesi* by the direct inoculation of blood from infected animals, in which

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\*The fact that on two occasions the injection of parasitised blood from a naturally infected specimen of *S. irus* apparently failed to produce an infection in *S. rhesus*, also suggests that isodiagnosis cannot be considered to be an infallible test. It is probable, however, that in the latter instances the failure was only apparent, not real, and that the result was due to an unduly prolonged incubation period [*vide* experiments (p) and (q), Mulligan and Sinton, 1933].

no parasites could be found in the peripheral blood, in spite of careful searches on several consecutive days.

**Experiment (36).** Monkey No. 153 (*S. rhesus*).

This normal monkey was inoculated intraperitoneally with 0.25 c.c. of blood from *S. rhesus* (No. 66). The latter animal had previously suffered from a pure infection with *P. knowlesi*, but no parasites had been detected in its blood for 2 days prior to inoculation.

**Result.**—The normal monkey (No. 153) developed a typical infection with *P. knowlesi* after an incubation period of 5 days.

**Experiment (37).** Monkey No. 169 (*S. rhesus*).

This normal monkey was inoculated intraperitoneally with 0.25 c.c. of blood from *S. irus* (No. 162), in which at the time of the experiment no parasites could be detected in the peripheral blood. This animal had shown no parasites at the daily blood examinations for 16 days previously.

**Result.**—The normal monkey (No. 169) developed a typical infection with *P. knowlesi* after an incubation period of 5 days.

**Experiment (38).** Monkey No. 167 (*S. rhesus*).

This normal monkey received an intraperitoneal injection of 0.25 c.c. of blood from *S. irus* (No. 103). The latter animal had previously shown a pure infection with *P. knowlesi*, but no parasites had been detected in its blood for 75 days, in spite of frequent examinations.

**Result.**—On 14th day after inoculation, the normal monkey (No. 167) showed parasites in the peripheral blood. A typical infection with *P. knowlesi* resulted.

**Experiment (39).** Monkey No. 160 (*S. irus*).

This apparently normal animal\* was given an intraperitoneal injection of 0.25 c.c. of blood from a specimen of *S. irus* (No. 162) found infected in nature. No parasites had been detected in the blood of the latter animal for 18 days prior to the date of inoculation, in spite of daily examinations.

**Result.**—A mild infection with *P. knowlesi* developed in the normal animal (No. 160) after an incubation period of 8 days.

**Experiment (40).** Monkey No. 171 (*S. rhesus*).

This normal animal was inoculated intraperitoneally with 0.25 c.c. of blood from *S. irus* (No. 56). No parasites had been detected in the blood of the latter animal for 12 days prior to the inoculation. Previously this monkey had shown a natural mixed infection with *P. knowlesi* and *P. inui* var. *cynomolgi*, the latter being the predominant species prior to the time of inoculation.

**Result.**—The normal monkey (No. 171) developed a typical infection with *P. knowlesi* 9 days after the inoculation.

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\*No parasites were detected in the blood of this animal by microscopical examination after protein shock.



**Experiment (41). Monkey No. 166 (*S. rhesus*).**

This normal monkey was inoculated intraperitoneally with 0.25 c.c. of blood from *S. rhesus* (No. 100). No parasites had been detected in the latter animal for 37 days prior to the time of inoculation. Previously this monkey had shown a pure infection with *P. knowlesi*.

**Result.**—No parasites were detected in the blood of the normal animal (No. 166) up to 40 days after the inoculation. It was, therefore, thought that the donor animal (No. 100) might be free from infection, but an acute relapse occurred in this monkey after splenectomy [*vide* experiment (12)].

The above experiments indicate clearly that monkey blood may prove to be infective to susceptible animals, even when many repeated, careful and protracted examinations of the peripheral blood have failed to reveal the presence of parasites. On the contrary the result of one experiment (No. 41) shows that 'isodiagnosis' is not an infallible test for latent infections. The latter finding is supported by the observations of Leger and Bouilliez (1913), quoted above. There is, however, much evidence to show that, while 'isodiagnosis' may occasionally fail, it is a test of very considerable delicacy for latent malarial infections in monkeys.

**(e) Splenectomy.**

Gonder and Rodenwaldt (1910) observed the effect of the removal of the spleen in monkeys, in which no parasites could be detected in the peripheral blood. They noted that after this operation, parasites reappeared in the peripheral blood and persisted there for a long time.

It has been our experience that splenectomy performed on monkeys with latent malarial infections is associated with a reappearance of parasites in the peripheral blood. This has been found in monkeys in which no parasites had been detected for long periods (as long as 3 months) in spite of careful and repeated blood examinations. In one case splenectomy caused the reappearance of parasites in a monkey in which the 'isodiagnosis' test had failed to demonstrate the presence of a latent infection [experiments (12) and (41)].

Splenectomy as a method for the detection of latent infections, although valuable, is not practicable on a large scale. Animals, which have proved to be uninfected by this method, cannot afterwards be used to study the usual pathogenic effects of any species of *Plasmodium* in the normal animal. This is a distinct disadvantage.

**(f) Routine methods now used in our experiments.**

There is no evidence to show that any of the methods described above for the detection of latent malarial infections in monkeys is infallible. There is, however, good reason to believe that for ordinary purposes the vast majority of such infections, if not all, will be detected by a combination of some of these procedures,

The method now employed as a routine in our experiments is as follows :—

The bloods of apparently healthy monkeys are examined by the thick-film method over long periods (usually daily for some weeks). If no parasites are detected, an intravenous injection of foreign protein (human blood or horse serum) is given, and the blood of the animal is again subjected to careful microscopical examination. If no parasites be detected within 24 hours after the injection of foreign protein, blood from the animal under test is then injected into a healthy animal. If no parasites have been detected, either in the blood of the original monkey or in the blood of the inoculated one within a reasonable period, it is presumed that the animal under test is not suffering from a malarial infection. We believe that this routine procedure is a more delicate and reliable test for the presence of latent infections than any other single method yet suggested.

Since this combined method was started, it has never failed to reveal the presence of parasites in any known latent infection. It has never given positive results in any specimen of *S. rhesus* which had not been infected experimentally. Several specimens of both *S. rhesus* and *S. irus*, found negative by these methods, have afterwards been splenectomized. In none of these was the operation followed by the re-awakening of a latent infection, nor did smears of the extirpated spleen show any Plasmodia.

This valuable combination of methods is, unfortunately, not practicable as a routine measure in human malaria.

## APPENDIX II.

### METHODS FOR THE ISOLATION OF PURE INFECTIONS OF *P. KNOWLESII* AND *P. INUI* VAR. *CYNOMOLGI* FROM MIXED ONES.

Most of the natural infections observed by us in *S. irus* have been mixed ones of these two species of *Plasmodium*. It has, therefore, been necessary to obtain pure infections with each species before any further studies on immunity could be carried out. A description of the various methods used for isolating pure infections are given below. Some of these may prove useful to workers who are confronted with the same problem in other localities.

#### (A) METHODS FOR THE ISOLATION OF PURE INFECTIONS WITH *P. KNOWLESII*.

##### (1) By sub-inoculation to normal susceptible animals.

No difficulty has been experienced in isolating pure infections of *P. knowlesi* in *S. rhesus*. The extreme susceptibility of the latter species of monkey to *P. knowlesi* makes it an easy matter to obtain by blood inoculation very heavy infections, in which the latter species of *Plasmodium* is the only one detectable. If, at the height of this infection, a small amount of blood (not more than 0.1 c.c.) be inoculated into a fresh specimen of *S. rhesus*, the resultant infection is often a pure one of *P. knowlesi*. More certain results are

obtained if a similar procedure be used to make several sub-passages in series, so that the chances of passing *P. inui* var. *cynomolgi* at the same time are further reduced. The greater the number of such sub-passages the greater are the chances of obtaining a pure infection.

**(2) By sub-inoculation combined with quinine treatment.**

While the above method has been found successful on many occasions, more certain and rapid results are obtained if it be combined with quinine treatment of the inoculated animal. It has been found, during the progress of this work, that in many cases the course of quinine treatment required to control *P. knowlesi* in mixed infections in *S. rhesus* is sufficient to eradicate *P. inui* var. *cynomolgi* completely. While in other cases, it has reduced the numbers of the latter parasite in the peripheral blood to such a low level that in sub-inoculations an infection with *P. knowlesi* only is passaged. By combining treatment with the previous method, it has been found comparatively easy to isolate pure infections with *P. knowlesi*. To ensure the purity of the infection, it is advisable to make two or three sub-passages in series to *S. rhesus*, using the same precautions of a small dosage of blood combined with adequate treatment of the inoculated animals.

**(B) METHODS FOR THE ISOLATION OF PURE INFECTIONS OF  
*P. INUI* VAR. *CYNOMOLGI*.**

The isolation of pure infections of this *Plasmodium* from mixed infections has proved much more difficult than in the case of *P. knowlesi*.

**(1) By sub-inoculation to normal susceptible animals.**

While the very severe infections, which are produced in *S. rhesus* by *P. knowlesi*, facilitate the isolation of the latter parasite, it is this feature which is responsible for the difficulty in isolating *P. inui* var. *cynomolgi*.

Many inoculations of very small amounts of blood have been made from naturally infected specimens of *S. irus*, when the predominant, and apparently the only, parasite in the peripheral blood was *P. inui* var. *cynomolgi*. On only one occasion have we been able to isolate a pure infection of the latter parasite by this method. In all the other trials, the more virulent *P. knowlesi* quickly predominated in the inoculated animal. It is possible that if *S. irus*, which possesses a considerable degree of tolerance to the latter parasite, were used, the results would have been more successful. Unfortunately, this species of monkey is not easy to procure in northern India, and many of the specimens obtainable already show natural infections.

As in the methods for the isolation of *P. knowlesi* by blood inoculation, *P. inui* var. *cynomolgi* should be sub-passaged as soon as the infection is detected.

The success of this method is largely a matter of chance, so it is necessary to consider some more reliable ones.

## (2) By mosquito transmission.

It has been pointed out earlier in this paper, that all attempts to transmit *P. knowlesi* by mosquitoes have so far been unsuccessful. This failure may be due to a scarcity of viable gametocytes in the peripheral blood of the host. It seems more probable, however, that the Anophelines used are not very susceptible to infection with this species of *Plasmodium*. These results resemble the recorded difficulties experienced in attempts to transmit *P. malariae*.

On the other hand, the transmission of *P. inui* var. *cynomolgi* by some of the common Indian Anophelines appears to be a comparatively easy matter. Advantage has been taken of these differences to obtain pure infections with *P. inui* var. *cynomolgi*. *A. annularis* (*A. fuliginosus*), which has proved very susceptible to infection with the latter parasite, has been used for this work.

Several malariologists have remarked upon the fact that, in mixed infections in human malaria, the common type of observation is to find the asexual forms of one species of *Plasmodium* associated with the sexual forms of the other. In *S. rhesus*, having mixed infections of *P. knowlesi* and *P. inui* var. *cynomolgi*, the predominant parasites are usually the asexual forms of the former parasite. On the other hand, the gametocytes of both species may be present. We have taken advantage of this fact in our mosquito-transmission experiments, and used such animals for feeding experiments.

### (a) Isolation from natural mixed infections.

The parasitic infections in naturally infected specimens of *S. irus* are usually so scanty that these animals are unsuitable for insect-transmission experiments. So far we have not been able to infect mosquitoes from such hosts. It is suggested, therefore, that the parasitic infections in the peripheral blood of these animals might be stimulated by either protein shock or by splenectomy, to aid such experiments. The further steps of such work would be similar to those with experimentally infected animals.

### (b) Isolation from experimental mixed infections.

When natural mixed infections are inoculated from *S. irus* into *S. rhesus*, *P. knowlesi* is the predominant parasite. If the life of the infected animal is to be saved, it is necessary to give treatment. The treatment given in these experiments is quinine. This is administered in minimal doses, so that there will be less chance of eliminating *P. inui* var. *cynomolgi* from the freshly infected animals. It is advisable to infect two or three animals, as some of them may die under minimal treatment.

When these animals have recovered from the acute infection, and a suitable number of gametocytes\* are found in the blood, specimens of *A. annularis* are

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\*It is often difficult to differentiate the gametocytes of the two species of *Plasmodium* with certainty. If, however, any animal with a mixed infection shows large numbers of this form of parasite, which readily exflagellate, mosquitoes are fed on the chance that a transmissible infection of the salivary glands will be obtained.

fed on the infected animal. These fed insects are kept under suitable conditions of temperature and humidity. Specimens are dissected periodically to determine the presence and progress of any infection which may develop. A few days after sporozoites have been detected in the salivary glands\*, the insects are allowed to feed on a normal specimen of *S. rhesus*. These feeds are repeated at intervals of a few days on several occasions.\*

On the one occasion in which this method was used, a pure infection of *P. inui* var. *cynomolgi* was obtained from a mixed infection in *S. rhesus*. Further work along these lines is contemplated, as we believe that this may eventually prove the easiest means of isolating pure infections of this parasite.

### (3) Isolation by immunization of *S. rhesus* against *P. knowlesi*.

It has been pointed out that one of the difficulties in obtaining pure infections of *P. inui* var. *cynomolgi* lies in the greater susceptibility of *S. rhesus* to *P. knowlesi*. It would, therefore, be logical to expect that, if blood inoculations were made from mixed infection into an animal highly immunized against the latter Plasmodium, a predominant infection with *P. inui* var. *cynomolgi* would be obtained. It might then be possible to isolate this infection by the passage of small amounts of blood, or by mosquito transmission.

Mulligan and Sinton (1933) have shown that a specimen of *S. rhesus* infected with a pure strain of *P. knowlesi*, is almost completely tolerant to superinfection with the same strain of parasite. Attempts are being made to utilize this method for the isolation of pure infections of *P. inui* var. *cynomolgi*.

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\* We have found that a certain degree of maturation appears necessary before sporozoites become infective. Several experiments were made in which sporozoites, obtained either from ruptured oöcysts or from fresh infected salivary glands, were injected into susceptible monkeys. The results were always negative, even when large doses of sporozoites were given a few minutes after extraction from the mosquito. This suggests that sporozoites, which have only been in the salivary glands for a short time, are not necessarily infective, but that some period of maturation is necessary. This possibility must be considered, if the 'new procedure for malaria research' advocated by James, Nicol and Shute (1927) is being used. A similar failure to obtain infection by the injection of sporozoites has been recorded by Green (1932). The latter author used the sporozoites of *P. inui* (? *P. inui* var. *cynomolgi*).

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# MIXED INFECTIONS IN THE MALARIA OF THE LOWER MONKEYS.

## Part II.

### THE PROBABLE OCCURRENCE OF MIXED INFECTIONS IN SOME OF THE OLDER RECORDS OF MONKEY MALARIA.

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## A. INTRODUCTION.

THE occurrence of mixed infections in human malaria is not only common but is much more frequent than is generally recognized. Knowles and Senior White (1930) have collected a large number of records of such infections from all parts of the world. They found that mixed infections constitute about 3.7 per cent of the general malaria of the world, as judged by the reports consulted, but they consider this figure is very much too low. 'Given a competent observer, examination of 100 fields of a thin film plus a reasonably careful examination of one thick film will enable a positive diagnosis to be made in 95 per cent of all cases of developed malaria; but it will only discover 67 per cent of cases of mixed infection'.\* Perhaps the most important factor of all 'in the diagnosis of malaria is the degree of training, powers of observation and experience of the investigator'. To this might be added in the case of mixed infections, the expert knowledge of the observer in relation to the various stages of the parasites and their differential diagnostic characters, as well as the number and duration of the examinations made. The published data on the morphology and specific characteristics of many of the different Plasmodia described from monkeys has been, and still is, very meagre and defective. Lack of such knowledge is probably responsible to a large extent for the failure to recognize mixed plasmodial infections in these animals in the past.

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\* This is borne out by our experience of blood preparations given to the students in our malaria classes. Workers and former students in various parts of India very kindly send us batches of 20 or more slides from single cases of malaria showing parasitic infections suitable for teaching purposes. When 20 students have examined slides from one patient, for 10-15 minutes in both thin- and thick-film preparations, it is astonishing what a large proportion of unsuspected, but undoubted, mixed infections are detected.

It is impossible to summarize here any large portion of the work which has been done on mixed infections in human malaria. A few examples which appear relevant to the points under discussion are quoted below :

Deekz and James (1911) state that 'in a series of experiments to determine the temperature curves in estivo-autumnal and in tertian malaria, we withheld quinine. Fifteen of these experiments were with what we thought at first to be unmixed tertian infections. Before the experiments were concluded, in eleven of these tertian infections we found either estivo-autumnal schizonts or crescents at some time in the course of the fever. In several, estivo-autumnal infections entirely replaced the tertian, but we did not observe the converse'.

These would appear to be mainly records of acute infections, and not of chronic cases relapsing after treatment, such as are mentioned below.

The work on malaria carried out during and after the Great War added much to our knowledge of mixed infections. Indeed these were so common, and so frequently undetected at one examination, that several well-known workers were convinced of the unicity of the human malaria parasites. Many observers have recorded the after histories of such unsuspected mixed infections.

Acton, Curjel and Dewey (1920) investigated a series of 102 cases previously diagnosed as undoubted infections with *P. falciparum*, because of the presence of crescents. When these cases, having been treated on the plains, came under observation at a convalescent depôt in the hills, 34 showed no parasites during 8 weeks of blood observation, while 64 relapsed with *P. vivax* in their bloods. In the original diagnoses only 7 mixed infections had been recorded among these patients. These workers also report that 13 patients, in whom *P. falciparum* was found originally by them, relapsed as benign tertian infections after the termination of treatment.

Similarly in the treatment centres supervised by workers at the Liverpool School of Tropical Medicine during the War and afterwards, the vast majority of the patients were found, on return to England, to be suffering from infections with *P. vivax*, although, in a large number of instances, the original diagnoses made abroad had been of infections with *P. falciparum* (Stephens *et al.*, 1917-1921).

Wenyon (1926) states that 'an individual infected with both *P. vivax* and *P. falciparum* may have an attack of malaria in which only the latter is evident, while at a later attack *P. vivax* appears. It frequently happens that persons become infected in malarious countries with both these forms, but that *P. falciparum* predominates to such an extent that it is alone detected. After they leave the malarious country, the *P. falciparum* infection gradually disappears. An attack of malaria occurs, and is found to be due to the more persistent *P. vivax*, which was not originally seen, and which in some way was suppressed by *P. falciparum* when it was actively multiplying'. Many cases diagnosed as malignant tertian malaria in the autumn, relapse as benign tertian in the spring.

These records suggest that the more virulent and shorter-lived infection, *P. falciparum*, tends to predominate in the primary acute attack, while the more chronic and benign infection, *P. vivax*, tends to become more prominent later. Such a sequence of events closely resembles our experience with mixed infections of *P. knowlesi* and *P. inui* var. *cynomolgi* in monkey malaria. The former parasite is much more virulent and has a 24-hour schizogony cycle. It, therefore, quickly becomes the predominant infection in susceptible animals like *S. rhesus*. On the other hand, *P. inui* var. *cynomolgi* is much more benign

in its effects and its schizogony only takes place every 48 hours. When mixed infections are injected into *S. rhesus*, the latter parasites can often be detected in very scanty numbers in the very early stages of the disease. The detection of these parasites was probably more frequent in such known cases of mixed infection than would normally be the case, because a special and prolonged search for such forms had usually been made. When, however, such mixed infections are present in *S. irus*, *P. inui* var. *cynomolgi* is usually much more evident in the early stages of the infection in our monkeys, because these animals in most instances seem to have a considerable degree of tolerance to *P. knowlesi*. Under these conditions the former parasite is not obscured to the same extent by the multiplication of the latter, and the stippling produced in the infested cells makes it more conspicuous (Sinton and Mulligan, 1933b.)

Another reason for this phenomenon is that in mixed infections the presence of the asexual forms of one species of parasite in the peripheral blood appears to be associated with the disappearance of the asexual forms of the other.

Wenyon (1926) states 'another important feature of mixed infections is that, though two or even three species of parasite are present, only one may be found. An individual infected with both *P. vivax* and *P. falciparum* may have an attack in which only the latter is evident, while at a later attack only *P. vivax* appears'. James (1931) points out that 'in primary infections a concurrent mixed infection with two species of malaria parasite cannot often be demonstrated, because one species quickly becomes predominant and the other disappears until the attack caused by the predominant species is over'. Knowles and Das Gupta (1932) also emphasize the fact that in mixed infections one species of parasite usually predominates at the expense of the other.

It seems to us very probable that, if the same care were taken to search for mixed infections very early in the primary attacks of human malaria, as was used by us in studying the parasite picture in known cases of mixed infection in monkey malaria, a very much higher percentage of mixed infections would be detected (Sinton and Mulligan, 1933b). Unfortunately, in routine blood examinations in medical practice in the tropics, such a painstaking investigation is usually impossible, on account of the time involved.

James (1931) found that, during a relapse of a chronic infection with *P. vivax*, the incubation period of a superinfection with *P. falciparum* was prolonged. He suggested, therefore, that the seasonal incidence of the different types of human malaria might have some relationship to the apparent antagonism between the different species of *Plasmodium*.

This worker remarks that 'if further experiment shows that there is really an antagonism between *P. vivax* and *P. falciparum*, it might provide a partial explanation of the different seasonal incidence of these two kinds of malaria. The idea is that if patients were infected with both the species at the same time, the clinical appearance of the attack due to *P. falciparum* would be delayed until the attack due to *P. vivax* had run its course or part of its course. In one of our patients who had a relapse of a previous benign tertian infection during the incubation period of an infection with *P. falciparum*, the primary attack due to the latter parasite did not begin until the benign tertian relapse ended. This made the incubation period of *P. falciparum* infection eighteen days instead of six or seven. Several objections to the idea can be advanced. Possibly the difficulty of getting two species to run

concurrently happens only when one of the infections is primary, the other secondary. We have mentioned the idea as a suggestion for study rather than as one on which we have formed an opinion'.

The experiments recorded by Sinton and Mulligan (1933*b*) show that, when the two simian species of *Plasmodium* are transmitted at the same time by blood inoculation, it is the more virulent species, *P. knowlesi*, which quickly predominates and causes acute symptoms. Under the ordinary conditions of routine blood examination, the more benign species, *P. inui* var. *cynomolgi*, may remain undetected in the early stages of an unsuspected mixed infection. The latter, however, becomes predominant at some later period, when the acute infection with *P. knowlesi* has died down, if the treatment needed to control the latter infection has not eliminated the former parasite completely (*vide infra*).

One might compare the more virulent human *Plasmodium*, *P. falciparum*, with *P. knowlesi*, and the more benign one, *P. vivax*, with *P. inui* var. *cynomolgi*. It appears very probable from the records of mixed infections in human malaria recorded above, that these behave in the same manner as do such infections in monkeys (*vide* Sinton and Mulligan, 1933*b*). The rapid appearance and predominance of *P. knowlesi* under these experimental conditions, lends no support to the suggestion of James (1931) that a much delayed appearance of *P. falciparum* may occur, when non-immune patients are infected simultaneously with this parasite and *P. vivax*. Antic (1925) has recorded the production of experimental mixed infections with these two human Plasmodia, and his results are similar to ours with simian malaria. It must, however, be remembered that these experiments were carried out by blood inoculation, and it may be that transmission through the insect host would produce different results.

James (1931) also notes a prolongation of the incubation period when *P. falciparum* was superinfected upon a chronic infection with *P. vivax*. There also seems to have been some prolongation of this period in the case reported by Sinton and Mulligan (1933*b*), where *P. knowlesi* was superinfected upon a chronic infection with *P. inui* var. *cynomolgi*. The prolongation of the incubation periods in these two instances appears much too short to have any appreciable influence in determining the seasonal prevalence of *P. falciparum*. It is possible, however, that the more marked prevalence of infections with the latter parasite at some seasons, as compared with *P. vivax*, may be to some degree more apparent than real. The acute infections with the former parasite may so suppress the latter that many mixed infections with *P. vivax* remain undetected, and are only apparent at a much later date. This would be in keeping with the results recorded during the War. It would also help to account for the more common occurrence of *P. vivax* in the spring and of *P. falciparum* in the autumn.

A third factor which must be considered in relation to the detection of mixed infections is the question of treatment. This has been discussed more

fully in an earlier paper (Sinton and Mulligan, 1933b). As mentioned previously, a large number of cases during the War, originally diagnosed as pure infections with *P. falciparum* and treated for this fever, showed in their relapses not this parasite but *P. vivax*. This is now recognized as being due to the fact that most pure infections with *P. falciparum*, and many primary infections with *P. vivax*, were cured by the courses of treatment which these malaria patients received before they were drafted to the malaria convalescent depôts. It was in the latter institutions that the vast majority of these observations were made. This preliminary treatment resulted in the elimination of most infections, except in those patients with some individual idiosyncrasy, or who were perhaps infected with strains of parasite which were resistant, for some unknown reason, to the permanent curative effects of quinine (Sinton, 1931).

A somewhat similar result has been observed in mixed infections in *S. rhesus*. Here the very acute infection caused by *P. knowlesi* necessitates early and vigorous treatment during the primary attack, and sometimes in early relapses also. It has been found (Sinton and Mulligan, 1933b) that this treatment, in some cases, completely and permanently eliminates the infection with *P. inui* var. *cynomolgi*, before this parasite has appeared in numbers capable of detection without prolonged and expert blood examination. If treatment be not given, the animals die rapidly of the infection with *P. knowlesi*, before the other species of *Plasmodium* has time to become predominant or conspicuous. Under such circumstances infections in *S. rhesus* usually appear to be pure, although they were not so originally\*.

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\* James (1931) reports that the therapeutic action of quinine in benign tertian malaria is considerably enhanced, if the patient be allowed to develop a certain degree of tolerance to the infection before treatment is commenced. He, however, remarks that 'it would be unjustifiable at present to withhold quinine from a case of malignant tertian malaria later than the first discovery of parasites in the blood'.

It appears to us that, in view of the not uncommon occurrence of unsuspected mixed infections in malarial patients in the tropics, one is seldom or never justified in allowing any malarial infection to remain untreated in the hope of obtaining this degree of acquired immunity. It was found by Antic (1925) that in experimental mixed infections with *P. vivax* and *P. falciparum*, the former parasite could be detected at a very early stage of the infection, but that the latter quickly predominated. The more conspicuous morphology of *P. vivax* and its presence in the peripheral blood during the whole of its schizogony cycle, make it much more easily detectable than the small rings of *P. falciparum* which are usually present in the circulating blood for a much shorter period of each cycle. The latter parasite might, therefore, be overlooked during the first few paroxysms of an acute attack. The difficulty which is sometimes experienced in detecting *P. falciparum* in some early acute cases has been noted by many workers, even when there has been no mixed infection to obscure the issue. If the patient remains untreated, in the hope that the more benign parasite may produce some tolerance to aid the effect of treatment, the unsuspected infection with *P. falciparum* may quickly predominate. Daily blood examinations are usually impossible under the conditions of general practice in the tropics. If treatment be withheld on the assumption that the primary diagnosis of *P. vivax* is correct, the rapid development of

It has been recognized for a long time that human malarial parasites may react very differently to the same therapeutic agents. Any precise knowledge about the action of drugs on the different Plasmodia of monkeys has, however, been wanting. The absence of such information has helped to prevent the recognition of mixed infections in these animals in the past.

## B. GENERAL CONSIDERATION OF MIXED MALARIAL INFECTION IN MONKEYS.

As has been noted above, the occurrence of mixed infections in human malaria is not an uncommon phenomenon. With the exception of the suggestion which was made and rejected by Knowles and Das Gupta (1932) that some of their results might be attributable to such infections, no other worker appears to have considered this possibility in monkey malaria.

There is no reason to suppose that there is only one species of *Plasmodium* confined to the monkeys of each different locality, or that each species of monkey is susceptible to infection with only one species of this parasite. The communal habits of the lower monkeys and the absence of any protective clothing should greatly facilitate the transmission of infections in nature. Such animals are not known to take any therapeutic measures against their infections, and the elimination of these appears, therefore, entirely dependent upon the natural defences of the body. The production of cure under such conditions would necessarily be slow, so that carriers of the *Plasmodium*, in a form infective for the insect host, would probably be common, especially among the younger animals. The conditions would probably be very similar to those seen in those remote villages in India, where hyperendemic malaria is present and where adequate specific treatment is comparatively seldom available. Under such conditions, a very large proportion of the younger individuals show clinical symptoms of malaria, with high parasite and gametocyte indices, while

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*P. falciparum* may remain undetected until therapeutic measures are too late to save the life of the patient, or at least to avert a pernicious attack.

Many of the older physicians recognized the occurrence of spontaneous recovery in the intermittent fevers of the spring and winter, and Torti recommended that these should often be left 'viribus naturæ et imminenti naturæ'. In the case of the autumnal fevers, however, the necessity for immediate treatment with cinchona was emphasized.

While believing that some degree of acquired tolerance probably augments the action of quinine, we do not think that patients can be left, as a routine measure, untreated so that such tolerance will develop. At least it does not seem a wise procedure in areas where *P. falciparum* is common. The shortage of hospital accommodation and expert medical supervision in many tropical areas also precludes this method as a routine step, however desirable it may be under the more carefully controlled conditions of therapeutic malaria.

The work summarized by Mulligan and Sinton (1933) also suggests very strongly that any immunity, thus acquired, may only be effective against the same strain of parasite, and will have no very marked effect if the patient be reinfected with another strain. Now that several of the new synthetic drugs have been found to have a marked curative action in chronic infections with *P. vivax*, the necessity for leaving malarial patients untreated in this fever seems to have disappeared to a very large extent.

- among the adult population clinical manifestations of the disease are rare, and parasites usually scanty in the peripheral blood. It has been shown in a previous paper (Mulligan and Sinton, 1933) that the infection of monkeys with one of the species of parasite under discussion produces little or no immunity against the other species. Under these conditions there seems to be no apparent reason why mixed infections should not occur, if both species of parasite and suitable insect carriers be present in the community.

It appears very probable that some such occurrence may be responsible, partly at least, for the very varied morphology and pathogenicity that have been attributed to some species of monkey *Plasmodium* considered in the past to be identical.

In a number of instances, the descriptions of these parasites have been based upon the findings obtained in experimental animals. This was usually because the natural hosts have shown very scanty parasites in the peripheral blood, and the infections have been of a chronic or latent character. When one considers the relative frequency with which natural infections appear to occur in several of the common species of monkey used in such experiments (*vide* Appendix), one cannot rule out the possibility that either the natural host, or the experimental animal, had an undetected latent infection with one or more species of *Plasmodium*. Under these circumstances in inoculation experiments, mixed infections might occur, due either to

(a) the inoculation of a pure infection from a natural host into an experimental animal having an undetected latent infection, or

(b) the inoculation of an undetected and unsuspected mixed infection from one animal host to another which is susceptible to infection with the injected parasites.

As has been discussed previously (Sinton and Mulligan, 1933b), it is very difficult to exclude the presence of such latent infections with any degree of certainty by the usual method of preliminary blood examinations. They are apparently not very uncommon in some species of monkey. Our experience has been that natural infections in *S. irus* may escape detection by microscopical examination of the blood during long periods, even when these are made daily. In some published studies on these Plasmodia, the occurrence of such infections appears to have been excluded, only because of negative blood findings recorded during a comparatively short period. If, under such conditions of experiment, the original infection be passed to a large number of different monkeys of varied species and genera, there seems to be a very great probability that any parasite which predominates, or is present, at the end of such a series of passages, would not necessarily be the same as that originally observed (*vide infra* Leger and Bouilliez, 1913). On the other hand, the Indian workers have been fortunate in having, easily available, large numbers of a species of monkey, *S. rhesus*, which is susceptible to two, if not three, of the Oriental types of simian *Plasmodium*. In addition, natural infections in Indian specimens of

this animal are apparently of extreme rarity, and are probably absent in those from northern India.

Although a scanty infection may be detectable in the natural host, it is often impossible to exclude the presence of a mixed one. This is especially the case when one considers how little is known about the precise differential characters of the various species of monkey Plasmodia.

The work of Knowles and Das Gupta (1932) has shown that the susceptibility of several species of monkey to infection with malarial parasites may vary very considerably. This has also been our experience and that of several other workers. It is difficult, in our present state of knowledge, to decide definitely whether this is due to a natural immunity in the animal species, or to a tolerance acquired as the result of previous infection. The evidence available favours the latter view.

This variability of susceptibility has an important bearing on the study of mixed infections. When the blood of animals having mixed infections is injected into other susceptible hosts, it is not necessarily the parasite which predominated in the original host at the time of injection, which becomes most conspicuous in the new host. This is especially the case if the inoculated animal belongs to a different species or genus, which may be either more susceptible or more tolerant to one species of parasite than to another.

Another factor is also introduced here. The injection of blood from a different species of animal may produce protein shock in the inoculated animal, and this may awaken an unsuspected latent infection. This is what occurred (a) when Berenberg-Gossler (1909) injected blood from an infected specimen of the American monkey, *Brachyurus calvus*, into *Silenus irus*, (b) when Knowles (1919) tried to infect *Pygathrix entellus* by means of infected human blood, and (c) possibly also in the experiments which are claimed to have produced successful transmission of human Plasmodia to the higher apes. In the first two instances, the inoculation did not cause any detectable infection with the parasite injected, but the protein shock caused by the foreign blood, awakened in the recipient animal a natural infection of another *Plasmodium*, which had previously been latent.

#### \*C. DISCUSSION OF SOME CHARACTERS REPORTED TO SHOW VARIATION IN THE MALARIAL INFECTIONS OF MONKEYS.

The variations recorded in relation to the malarial parasites of the lower monkeys have chiefly been concerned with (a) the morphology of the parasites, (b) with alterations in the infested red cells, and (c) with pathogenic effects in the same or other species of monkey. These changes are said to occur (i) after the infection has been transmitted from the natural host to other experimental animals, sometimes of the same and sometimes of other species or genera, or (ii), in some instances, after the infected animal has been splenectomized.



## I. CHANGES IN THE MORPHOLOGY OF THE PARASITE.

## (a) AFTER TRANSMISSION OF INFECTION TO OTHER HOSTS OF THE SAME OR OF DIFFERENT SPECIES.

Gonder and Berenberg-Gossler (1908) and Berenberg-Gossler (1909) report considerable morphological changes when the natural malarial infection, discovered by the former authors in *Cercocebus fuliginosus*, was injected into young animals of the same species. Knowles and Das Gupta (1932) have recorded an almost complete transformation in the morphology of a parasite which they found in *S. irus*, when the infection was inoculated into other Anthropoidea of different genera and species from the original host. Taliaferro (1932) has recorded similar changes in the malarial parasites of the Panamanian monkeys.

Berenberg-Gossler (1909) considers that the changes reported by him are due to the sensitivity of simian malarial parasites to the natural conditions of the body of the host. Grigorieva (1929) also thinks that the morphology of these parasites may be determined by similar conditions.

Sinton and Mulligan (1933b) carried out an extensive series of experiments to determine the cause of the apparent changes in morphology recorded by Knowles and Das Gupta (1932), in the Plasmodia found in a natural infection in *S. irus*. They were unable to detect any morphological variations when pure infections were studied. The conclusion reached by them was that the apparent pleomorphism of the parasites was due to undetected mixed infections.

## (b) AFTER SPLENECTOMY.

Berenberg-Gossler (1909) and Gonder and Rodenwaldt (1910) reported that, after splenectomy, the Plasmodia in their animals showed extraordinary division forms, which they considered to be degenerate. These changes were so great that Berenberg-Gossler thought, at first sight, that he was dealing with a new species of parasite.

Blanchard and Langeron (1913) state that, in splenectomized animals, the parasites studied by them became very amoeboid. Both the protoplasm and the nucleus were broken up into long threads in some instances, and the number of merozoites increased from 8 to 16. On the other hand, Bouilliez (1913) and Leger and Bouilliez (1913) state that no such changes occurred in the monkeys splenectomized by them.

Sinton and Mulligan (1933b) splenectomized a number of specimens of *S. rhesus* and *S. irus*, infected with *P. knowlesi* and with *P. inui* var. *cynomolgi*. In no case were they able to find that this operation caused any change in the characters of the species of *Plasmodium* responsible for the infections.

## (c) SUMMARY.

Various workers have reported definite morphological changes in different species of *Plasmodium* after passage to new hosts or after splenectomy of the

host. We have been unable to find any experimental evidence to confirm these findings in the case of the Plasmodia of Oriental monkeys, if a pure infection with only one species of parasite be studied.

It appears probable that most of the recorded changes were the result of unrecognized mixed or latent infections. The published data, upon which these changes were recorded, have been analysed later in this paper. There is very considerable internal evidence in these reports to support the view that unsuspected mixed infections were present in some, if not all, the experiments.

## II. ALTERATIONS IN THE STAINING REACTIONS OF THE INFESTED RED BLOOD CELLS.

### (a) AFTER TRANSMISSION OF THE INFECTION TO OTHER SPECIES OF ANIMAL, OR TO DIFFERENT SPECIMENS OF THE NATURAL HOST.

Mayer (1907, 1908) found considerable variations in the staining properties and frequency of the stippling in the infested red blood cells of the different animals used in his experiments. Gonder and Berenberg-Gossler (1908) and Berenberg-Gossler (1909) recorded enlargement and stippling of the infested red cells in artificially infected monkeys; these altered erythrocytes were sometimes seen in great numbers. On the other hand, in natural infections they only observed the phenomenon in two cells.

Knowles and Das Gupta (1932) reported marked stippling in the parasitized cells in the malarial infections studied by them in *S. irus*, while in specimens of *S. rhesus* infected from these animals this phenomenon was not seen.

### (b) IN INFECTIONS IN SPLENECTOMIZED ANIMALS.

Blanchard and Langeron (1913) reported that some of the Plasmodia observed in splenectomized monkeys showed long undulating filaments of chromatin. Although Leger and Bouilliez (1913) state that they found no changes in the morphology of the parasites after splenectomy, it is noteworthy that in their protocols the only two occasions upon which stippling is mentioned are after splenectomy.

Sinton and Mulligan (1933b) studied infections with both *P. knowlesi* and *P. inui* var. *cynomolgi* in *S. irus* and *S. rhesus* after splenectomy. These animals were splenectomized either before the infections were transmitted by blood inoculation or after the animals had been infected for some time. In no instance were they able to detect any change in the character of the stippling, when pure infections were studied.

### (c) DISCUSSION.

Considerable discussion has taken place concerning the constancy of the stippling produced by different species of simian Plasmodia. Some workers have found that such changes are almost constant, while other workers report that they are absent, or, if present, their occurrence is very irregular. The latter results have been recorded by competent observers, who had made special

efforts, and used special techniques, in their attempts to demonstrate such changes. These divergent opinions have been expressed about parasites, which had been identified as the same species, in several instances.

Although no other workers have recorded distinct stippling with *P. kochi* or any of its varieties,\* Gonder and Berenberg-Gossler (1909) and Berenberg-Gossler (1908) report this phenomenon in experimental infections with a parasite which they thought to be *P. kochi* (Lav.).

Halberstadter and Prowazek (1907) were unable to show stippling of the red cells in infections with *P. inui* (sens. restr.), in spite of the fact that they were able to demonstrate this change in infections with *P. pitheci*, studied at the same time. Mathis and Leger (1911), working with an infection, which they considered to be due to a pure strain of *P. inui*, noted that stippling was sometimes seen. At other times, and for no apparent reason, they were unable to demonstrate these changes.

Leger and Bouilliez (1913) observed stippling in a relatively small number of cases of monkeys of different species infected experimentally with what they thought was *P. inui* (sens. restr.). In such cases they were able to demonstrate these changes in the red cells with either Leishman's or Giemsa's stain. However, in the later stages of their experiments they were unable to show stippling even when Pappenheim's panoptic stain was used.

Mayer (1907, 1908), in infections with *P. inui* var. *cynomolgi*, found that in some animals he was able to show stippling, even after very weak staining with Giemsa's stain. On the contrary, in some other animals this change was much less evident, even when a strong stain was used. Blanchard and Langeron (1912, 1913) demonstrated stippling in a constant manner in an infection which they considered due to *P. inui* var. *cynomolgi*.

In spite of the different findings reported with *P. inui* (sens. restr.) Halb. and Prow., 1907, and with *P. inui* var. *cynomolgi* Mayer, 1907, several workers consider that the latter name is a synonym for the former parasite.† These varied reports have led to considerable discussion.

Mathis and Leger (1911) think that stippling is not a feature of primary importance in the specific differentiation of the Plasmodia of monkeys. They believe that its demonstration depends upon the stain used. Leger and Bouilliez (1913) adopt a similar attitude. On the other hand, Blanchard and Langeron (1912) attach the greatest importance to the occurrence of stippling as a diagnostic character. They believe that, if it be not demonstrable in certain infections, or at certain times, the failure is largely due to errors in technique, or attempts made to show it in old smears. Macfie (1928) apparently

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\* This refers to *P. kochi* as defined by Sinton and Mulligan (1932).

† We believe that *P. inui* var. *cynomolgi* is almost certainly a distinct species from *P. inui* (sens. restr.). As we have had no opportunity of studying the latter species experimentally, we have in the meantime retained the former species as a variety of the latter.

considers the presence or absence of this phenomenon important, if special attempts and appropriate technique have been used to demonstrate it.

Our experience with *P. inui* var. *cynomolgi* has been that there is no difficulty in demonstrating stippling of the infested red cells, when the parasite has reached the age of about 9 hours or more. We have found it as easy, and possibly easier, to detect than Schüffner's dots in infections with *P. vivax*. The stippling with *P. inui* var. *cynomolgi* is shown by ordinary methods, such as the use of either Leishman's or Giemsa's stain, without any special adjuvant technique. By special methods, such as a panoptic one where both these stains are used, stippling can be demonstrated at an even earlier stage in the schizogony cycle of the parasite. Green (1932) also finds this phenomenon constantly present in infections with *P. inui* var. *cynomolgi*.

With *P. knowlesi*, on the other hand, the stippling is more difficult to demonstrate and appears at a relatively later stage of development. When the ordinary technique of either Leishman or Giemsa is used, it may not be evident, or only faintly apparent in some cells. When, however, more prolonged staining is given, this change is in some instances more conspicuous. With a panoptic method (Leishman-Giemsa), it can be constantly demonstrated, if the parasite be at the proper stage of development.\* It is most evident in erythrocytes infested with schizonts in the presegmenting stage (*vide* Sinton and Mulligan, 1933a, Plate V). It is not so prominent, nor are the dots so clear-cut, as with *P. inui* var. *cynomolgi*.

We have found that stippling of the infested cells can always be demonstrated at certain stages of parasitic development in pure infections with either of these parasites, particularly if special methods are used. This fact makes us consider the character to be of primary importance in the specific diagnosis of the Plasmodia of monkeys.

It appears to us that the very diverse opinions expressed by expert workers can be reconciled, if one take into account the possibility that, in several instances, mixed infections were being studied. In some records, reference to the occasional occurrence of stippled cells suggests a mixed infection with *P. inui* var. *cynomolgi*, or with some other Plasmodium present at the stage of schizogony when stippling is demonstrable. The fact that in *P. knowlesi* infections such alterations are only a relatively transient phenomenon, and only demonstrable constantly by special staining methods, also suggests that in several instances some such parasite may have been present. The common occurrence of these two parasites as mixed infections in Oriental monkeys adds strong support to such an explanation. The reports of the different workers mentioned above have been analysed more fully from this point of view at a later stage of this paper.

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\* Knowles (1932a) also noted with a monkey malaria parasite, which seems to have been *P. knowlesi*, that 'stippling is not apparent with the usual methods of staining, but a very fine stippling, resembling Ziemann's stippling, can be demonstrated by Shute's technique'.

The curious filamentous 'protrusions of chromatin', described by Blanchard and Langeron (1913) in connection with adult parasites in splenectomized animals, appear to be related to stippling. We have seen in intact animals similar appearances in cells infested with adult schizonts of the same parasite, *P. inui* var. *cynomolgi*. They were observed most commonly when the staining was especially deep, or when a special effort had been made to demonstrate stippling intensely. When, however, other blood slides taken at exactly the same time were stained more lightly, these filamentous bodies were not seen or only very faintly. At the same time the nuclear chromatin was clearly demonstrated in these lightly stained specimens. This indicated that, although the filaments had taken on a stain resembling chromatin in colour, they were probably not of this nature. There seems little doubt that the 'nuclear filaments' reported by Blanchard and Langeron (1913) were not nuclear in origin, in most if not all cases. They probably represented linear agglomerations of intensely stained granules of stippling in the infested cells. A similar phenomenon, but of a less filamentous character, is occasionally seen around the segmenting forms and gametocytes of *P. knowlesi*, when intensive staining has been used. This appearance seems very similar to the capsule sometimes seen around crescents of *P. falciparum* and also around mature schizonts and gametocytes of some other Plasmodia, when the staining is intense. The latter phenomenon is also recorded by Flu (1908) with *P. inui* var. *cynomolgi*.\*

Mayer (1907, 1908) suggested that stippling depends not only on the species of parasite but also upon the individual characters of the host. We have studied this phenomenon with pure infections of both *P. knowlesi* and *P. inui* var. *cynomolgi* in several different species of monkey, and at all stages of infections both acute and chronic (Sinton and Mulligan, 1933b). We agree that the production of stippling appears to be a specific character of the Plasmodium, but have found no evidence that it is influenced by any individual characteristic of the host. Its time of appearance and its morphology with each species of parasite remained constant in all the animals studied by us. Neither the individuality of the host nor the degree of immunity developed by it appeared to have any detectable influence upon these features.

#### (d) SUMMARY.

Very divergent opinions have been expressed by different workers, with regard to the constancy of the occurrence of stippling in the infested red cells in infections reported to be due to the same species of parasite. Some workers have found this phenomenon so inconstant that they consider its presence or absence cannot be considered of much diagnostic importance.

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\* Garnham (1933) and Thomson (1933b) have recently described curious bodies found in erythrocytes infested with immature gametocytes of *P. falciparum*. It seems possible that these bodies may be of a similar nature to those under discussion.

Stippling has been found by us to be a constant feature of certain stages of the schizogony cycle of both *P. knowlesi* and *P. inui* var. *cynomolgi*. We, therefore, consider its presence or absence to be of primary diagnostic importance, when appropriate steps have been taken to demonstrate it at the proper period of the schizogony cycle. The varied results recorded by other workers appear to have been due, in some instances at least, to the presence of unrecognized mixed infections, and in others to a confusion of two different species of parasite.

### III. VARIATIONS IN THE PATHOGENICITY OF THE SAME SPECIES OF *PLASMODIUM* IN MONKEYS OF THE SAME OR DIFFERENT SPECIES.

It has been suggested by some authors that the degree of pathogenicity, produced in different species of monkey, might form a useful method for the differentiation of the various species of monkey Plasmodia. A study of the very divergent reports on pathogenicity, which have been published in the past, would appear to show that this feature of the infections would be of little aid in diagnosis.

Most workers have been struck with the very slight degree, or the complete absence, of detectable clinical symptoms in monkeys with natural malarial infections (*vide* Sinton and Mulligan, 1932, 1933a). The parasitic infection in such animals is usually so scanty that it has only been after inoculation of the infection into a susceptible animal, that a more detailed study of the parasites has been possible in many instances. It is chiefly in such artificial infections that marked variations in pathogenicity have been recorded. These differences have been reported not only in infections believed to be due to the same species and strain of parasite, but also when such parasites were injected into the same species of monkey.

Blanchard and Langeron (1912, 1913) studied a malarial infection in *S. irus*, which they believed to be a pure one of *P. inui* var. *cynomolgi*. In their first paper, they report that 1 out of 3 specimens inoculated by them died in 12 days, of a very acute infection resembling a pernicious attack in human malaria. In their second paper, 1 out of 7 monkeys of the same species died of an acute infection in 16 days, while the others survived for many weeks or months with chronic infections. They thought that these divergent results might be caused by individual susceptibility in the animals used, possibly as the result of varying degree of tolerance due to previous infections in nature.

In marked contrast with the acute infections encountered by Blanchard and Langeron (1912, 1913), are the cases reported by Mayer (1907, 1908) and Flu (1908). These workers observed few or no clinical manifestations, when the same species of *Plasmodium* was inoculated into *S. irus*. Our experience with pure infections of *P. inui* var. *cynomolgi* has been similar to that of the last workers. Even in *S. rhesus*, an animal which is very susceptible to *P. knowlesi*,

few or no ill effects appear to be caused by an infection with the former species of parasite. Green (1932) reports bouts of fever followed by splenic enlargement, and perhaps anæmia, in experimental infections in young specimens of *S. irus*.\*

Leger and Bouilliez (1913) passaged an infection which they state to have been due to *P. inui* (sens. restr.) through a very long series of lower monkeys. The pathogenic effects recorded are very varied, even when the same species of animal was used.

Napier and Campbell (1932), Knowles and Das Gupta (1932) and Sinton and Mulligan (1933a) found that blood from a natural malarial infection in *S. irus* produced few or no symptoms when inoculated into the same species of monkey. On the other hand, it produced a very severe and fatal infection, often with hæmoglobinuria, when injected into *S. rhesus*.

These great variations in pathogenicity recorded with the same species of parasite may be due to one or more of the following causes:—

- (a) variations in the tolerance or susceptibility of different monkeys,
- (b) variations in the virulence of the strain of parasite, as the result of animal passage, or
- (c) an unsuspected and undetected change in the strain or species of *Plasmodium* transmitted by blood inoculation.

#### (a) VARIATIONS IN THE IMMUNITY OR SUSCEPTIBILITY OF DIFFERENT MONKEYS

Variations in the immunity or tolerance of different animals might account for some of the differences in pathogenicity recorded. The degree of resistance to pathogenic effects present in any animal may depend either (i) upon an immunity, natural to the individual, the species or the genus, or (ii) upon an immunity or tolerance† acquired as the result of previous infection.

##### (i) Natural immunity.

It has been found that the higher apes possess an immunity, which appears to be natural, to infection with some of the *Plasmodia* of the lower monkeys (Halberstadter and Prowazek, 1907; Leger and Bouilliez, 1913). Knowles and

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\* This author was probably dealing in some cases with a mixed infection of *P. knowlesi* and *P. inui* var. *cynomolgi* (vide Sinton and Mulligan, 1933a).

† The 'tolerance' in plasmodial infections may be manifest in two different ways—(1) by few or no pathogenic effects in the presence of a comparatively heavy parasitic infection, or (2) by the power of the organism to keep the parasitic infection at such a low numerical level that no pathogenic effects are produced (Sinton *et al.*, 1931).

These are two quite distinct phenomena. The first is due to a tolerance to the effects of the special 'malarial toxin' produced by the strain or species of parasite causing the infection, the latter to some destructive action on the parasites themselves. These two phenomena are not necessarily present at the same time in equal degrees of activity.

Das Gupta (1932) managed, however, to produce a mild experimental infection in *Hy. hoolock* (the gibbon) after inoculation with *P. knowlesi*.

The latter workers also report the successful transmission of the same parasite to three men, but Berenberg-Gossler (1909) and Gonder and Rodenwaldt (1910) failed to infect man with *P. inui* var. *gonderi*. Clark and Dunn (1931) obtained a doubtful result with the American parasite, *P. brasilianum*. All attempts to infect the higher apes with human malaria parasites, and *vice versa*, have been unsuccessful, except for the unconfirmed record of Mesnil and Roubaud (1920).

As can be seen from the data collected by Sinton and Mulligan (1932, 1933a), many of the other attempts to transmit certain of the simian *Plasmodia* from one genus of host to another have failed. Some of these results may have been due to the presence of some acquired tolerance in the animals inoculated. There appears, however, to be no doubt that some species of Primate host have a complete natural immunity to certain *Plasmodia*, found as natural infections in other species.

Such a complete immunity would not affect the point under discussion, except in so far as such immunity is specific for one species of *Plasmodium*. If, therefore, during a series of passages an animal be found infected with a parasite to which it is normally immune, one should always suspect that another species of *Plasmodium* had been introduced at some stage of the experiment, or that the identification of the species of parasite is not correct.

The degree of natural immunity in individuals of different species of monkey to infection with different species of *Plasmodium* may not however be absolute in some instances. These variations may give rise to differences in the pathogenic effects of the same species of parasite in such animals. Thus *P. inui* var. *cynomolgi* gives rise to comparatively mild pathogenic effects in *S. irus*, *S. rhesus* and *S. sinicus*. The effects are more probably due to the naturally benign character of this *Plasmodium* rather than to any natural immunity in the species of animal inoculated. On the other hand in our work, *P. knowlesi* has shown very slight pathogenic effects in *S. irus*, much more marked ones in *S. sinicus*, and extremely severe and usually fatal manifestations in *S. rhesus*. These varying degrees of pathogenicity are probably to some extent the results of different grades of natural immunity or tolerance, either individual or specific. However, in the case of the very mild infections in *S. irus*, this natural immunity has probably been reinforced in our animals by some immunity or tolerance, acquired in nature as the result of earlier infections with the same species of parasite.

#### (ii) Acquired immunity or tolerance.

As many of the species of monkey used in experimental work have come from localities where natural malarial infections are not uncommon, it is very difficult to assess how much of any apparent tolerance is natural, and how much



has been acquired as the result of previous infection in nature. As was pointed out by Blanchard and Langeron (1912), one is completely ignorant of what are the pathogenic effects produced by these malarial infections in nature, and of the number of deaths they may cause in such conditions.

Kossel (1899) reports that parasites are rarely seen in older monkeys. Gonder and Berenberg-Gossler (1908) found few clinical symptoms in natural infections studied by them, but transmission of the infection to young animals produced a heavy parasitic infection. Macfie (1928) reports severe clinical symptoms in a natural infection in a young baboon. Clark (1930, 1931) has pointed out that among the monkeys of Panama, the infections are most prevalent in infant and juvenile specimens (*vide* Appendix). Green (1932) states that he found natural infections only in young specimens of *S. irus* in Malaya. Our experience with this species of monkey has been similar to that of Green.

None of the infected monkeys observed by Seidelin and Connal (1914) showed any clinical symptoms. These workers have suggested that in nature monkeys may show similar conditions to those seen among the local inhabitants in West Africa, *i.e.*, 'where practically all individuals are infected with malaria at an early age, thus acquiring for life a more or less marked immunity'.

It seems very probable that most of the species of monkey, which have been found to have high infection rates in nature (*vide* Appendix), acquire infection in early life. Under such circumstances, the young animals either die or develop a more or less effective tolerance to the pathogenic manifestations of the disease. In some cases this tolerance may break down under the stress of adverse circumstances, or when the animal is reinfected with a heterologous strain of parasite. As the animals increase in age, they probably acquire, by continued reinfection, a tolerance to all the local strains and species of parasite (Mulligan and Sinton, 1933).<sup>\*</sup> Whether continued weeding-out of the more susceptible animals by deaths from acute infection gradually tends to produce a race of animal which is born with a certain amount of natural immunity is uncertain. Such a state of affairs seems very possible.

One seldom sees very young monkeys in laboratory work, unless these be captured locally. This is probably because the vicissitudes of capture, confinement and transport kill off the weaklings. Such adverse conditions probably

<sup>\*</sup> Such a condition of tolerance in some species of monkey is probably very similar to that recorded by Cuica, Ballif and Vieru (1930), during their work on therapeutic malaria in a country where malaria is endemic (Roumania). These workers found that the proportion of patients who reacted with fever and parasites, after the primary inoculation with infected blood, was only 53·3 per cent in the case of *P. vivax*, 61·3 per cent in the case of *P. malariae*, and 80 per cent in the case of *P. falciparum*. A certain number of patients showed a temporary appearance of parasites in the peripheral blood and no fever (*P. vivax*—15·6 per cent; *P. malariae*—15 per cent; *P. falciparum*—18 per cent). This partial immunity or tolerance, as the result of previous infection in nature, appears to be very similar to that seen in our specimens of *S. irus*.

reactivate any latent infections in animals with slighter degrees of tolerance, and death may result during the relapses in many instances, because of the unnatural conditions of early captive life. It seems probable, therefore, that the majority of animals used in laboratories, in temperate climates at least, are those which have either had an infection, or have developed a considerable degree of tolerance to any malarial infection previously contracted. Some of these animals, if kept under conditions which preclude reinfection, will probably in time lose much of their acquired tolerance, as the infection dies out naturally (*vide infra* Blanchard and Langeron, 1912).

It is very difficult to determine how much of the tolerance, shown by some species of monkey in the laboratory, is due to natural immunity and how much to tolerance acquired as the result of previous infection. Apart from the record of Anderson and Cowdry (1928) that *P. kochi* var. *bouilliezi* can be transmitted to a monkey 'closely resembling *Callithrix personata*', an American species, no successful blood transmission experiments have been recorded with *P. kochi* or any of its varieties.\* These attempts have been unsuccessful even when made into animals of the same species as the original host. These findings add considerable support to the suggestion that much of the immunity or tolerance shown by such animals is the result of antecedent infections.

Many of the experiments on the transmission of the Oriental simian Plasmodia to monkeys from the same region have resulted in very mild or transient infections, even when the same species of animal was used as that showing the natural infection. Thus, when either *P. knowlesi* or *P. inui* var. *cynomolgi* are inoculated into *S. irus*, the clinical effects are slight, but when injected into *S. rhesus* they are more marked. The former animals had had, however, a chance of acquiring a tolerance previously in nature, which had not been the case with animals of the latter species from northern India. Although it is impossible to rule out some degree of natural immunity, yet it is very probable that much of the tolerance shown by animals coming from areas where simian malaria is endemic, is the result of previous infection.

The degree of tolerance to pathogenic effects, shown by any individual animal, may depend upon the strain of parasite with which it is reinfected, and upon the number of previous superinfections which it has had with heterologous strains. The work of Mulligan and Sinton (1933) and of Sinton and Mulligan (1933a) has shown that a primary infection with *P. knowlesi* will produce a disease, which is almost invariably fatal in *S. rhesus*. If, however, the life of the animal be saved by treatment, superinfection with the same strain of parasite will produce at most a very mild attack. If the animal be then superinfected with a heterologous strain of the same parasite, an acute attack will again develop, but this is apparently less severe than the primary one. Recovery from these attacks appears to be accompanied by some slight increase of

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\* This is *P. kochi* and its varieties as defined by Sinton and Mulligan (1932), and does not include the '*P. kochi*' of Gonder and Berenberg-Gossler (1908), which the former authors consider to be a distinct species, *P. inui* var. *gonderi*.

tolerance to the pathogenic effects of superinfection with other heterologous strains. These findings suggest that much of the tolerance to *P. knowlesi*, observed in our specimens of *S. irus*, may be attributable to similar infections and reinfections in nature, and that the animals usually seen in laboratory practice are the survivors of such infections.

Although apparently healthy animals may show a high degree of tolerance to the clinical effects of natural and experimental infections, it would seem that the protection is only relative in some instances. This tolerance may be diminished to varying extents by different adverse conditions, such as the presence of another disease, protein shock, traumatism, splenectomy, pregnancy, etc., and the infection may be reawakened by such conditions (Sergeant, 1908; Berenberg-Gossler, 1909; Blanchard and Langeron, 1913; Knowles, 1919; Grigorieva, 1929; Clark, 1930, etc.).

It appears very probable, therefore, that many of the experimental animals used in the study of simian malaria have had a prior infection. Unless one could obtain animals bred in an area free from monkey malaria, or which had lost their acquired tolerance as the result of cure, either natural or therapeutic, the pathogenicity test for the differentiation of *Plasmodia* does not seem practicable. The specimens of *S. rhesus* found in northern India would appear to fulfil these conditions. One species of monkey would not be sufficient, however, for universal use in determining the numerous permutations and combinations of pathogenicity required in the identification of large numbers of different species of *Plasmodium*.\*

#### (b) CHANGES IN VIRULENCE OF A STRAIN OF PARASITE AFTER ANIMAL PASSAGE

It has been found that changes in virulence may occur in some of the other pathogenic protozoa after passage through experimental animals. The possible occurrence of such changes in human malarial parasites was discussed in a previous paper (Mulligan and Sinton, 1933). It has been suggested that some such change may account in part for the varied degrees of virulence, which have been reported in some of the experimental work on monkey malaria.

Leger and Bouilliez (1913) state that the virulence of their infection neither diminished nor increased during 17 direct passages made by them. This statement would not appear to be borne out by their protocols, but, as discussed later, such apparent changes may have been due to the introduction of another species or strain of parasite.

Napier and Campbell (1932) and Knowles and Das Gupta (1932) believe that the virulence of the malarial infection studied by them has been enhanced

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\* It has been shown by Mulligan and Sinton (1933) that an infection with one strain of *Plasmodium* does not protect against superinfection with a different one. The cross-immunity experiments, which have been used to differentiate some of the other pathogenic protozoa of the blood, are not, therefore, applicable to the same extent in the case of *Plasmodia*.

by repeated blood passage through a very susceptible host, *S. rhesus*. The predominant, and probably the only, parasite in most of these passages was apparently *P. knowlesi*.

We have been unable to arrive at any definite conclusions on this point from our work with pure strains of the same parasite. *P. knowlesi* produces such very virulent effects in *S. rhesus*, that it is difficult to be certain of minor changes of pathogenicity in primary infections. As was pointed out in a previous paper (Sinton and Mulligan, 1933b), there appears to be some slight evidence that one of our strains of *P. inui* var. *cynomolgi* may have increased in pathogenicity under such conditions of passage. This may be due to the effects of 'acclimatization' of the parasite to a species of host other than the original one.

Most of the authentic instances of changes of virulence in protozoa after passage have been the result of many passages, not of a single one. Some of the variations reported by workers with monkey malaria appear to have been too sudden to be explicable on such an hypothesis only.

(c) VARIATIONS IN PATHOGENICITY DUE TO THE UNSUSPECTED INTRODUCTION OF A NEW STRAIN OR SPECIES OF *PLASMODIUM*.

While different degrees of tolerance, or changes in virulence, may account for the records of varying pathogenicity reported, in many instances these have been too divergent to be explicable upon these hypotheses alone. As will be seen when the data given by different workers are analysed later in this paper, some of the recorded variations are almost certainly due to the presence or unsuspected introduction of a different strain or species of parasite.

This change in strain or species may occur as the result of—

- (i) the presence of a mixed infection, natural or experimentally produced, in one of the animals used as a donor of infection, or
- (ii) the presence of a latent infection in one of the recipient animals.

(i) **Mixed infections.**

Mixed infections may be present in the donor animal either (a) as a natural mixed infection, or (b) as one experimentally produced by the inoculation of such natural infections into animals previously uninfected, or (c) by the inoculation of one species of parasite into an animal already having an infection with another species of *Plasmodium*.

When such mixed infections are inoculated into a fresh host no detectable infection may occur, if the animal already possesses an effective immunity against both strains of parasite inoculated. If, however, this immunity be not equally effective against both parasites, an infection with only one species may occur, or may predominate to such a degree as to be detectable. The latter species may not necessarily be the one which predominated in the original host.

Under these conditions, if the mixed nature of the infection be not recognized, very considerable differences may be reported in the pathogenicity and morphology of what appeared to be the same species of parasite. This is

apparently what occurred when undetected mixed infections of *P. knowlesi* and *P. inui* var. *cynomolgi* were injected into normal specimens of *S. rhesus*. Although the latter parasite was the one which predominated and caused few pathogenic effects in the natural host, *S. irus*, it was the former parasite which predominated and caused fatal results in the naturally non-immune species, *S. rhesus*.

Sub-passages from a non-immune animal injected with a mixed infection may result in the transmission of a species of parasite, which is not necessarily the one which predominated in the experimental animals previous to this passage. This is especially the case if therapeutic measures have tended to eliminate temporarily at least the less predominant parasite in the non-immune animal. This effect is shown by the fact that the passage of mixed infections from *S. irus* to *S. irus* has resulted in a predominant infection with *P. inui* var. *cynomolgi* in these animals. On the other hand, after passage from the latter animals to *S. rhesus*, *P. knowlesi* predominated and became, after treatment, the only detectable parasite during subsequent sub-passages (Sinton and Mulligan, 1933b).

#### (ii) Latent infections.

When either a pure or a mixed infection is passaged to an animal already having a latent infection with another species of *Plasmodium*, the parasites which predominate in the recipient animal may be either one of the injected species or be the parasite which was latent. The infection in the latter case was probably stimulated by the protein shock caused by the injection of foreign blood (Berenberg-Gossler, 1909; Knowles, 1919; Sinton and Mulligan, 1933b).

When the infection is again passaged from this secondary host, the parasite which appears most prominently in the next host will depend (a) upon the tolerance of this animal to the different species of *Plasmodium* injected, and (b) upon the species of parasite which is viable in the blood of the donor at the time. If the animal used in the latter passage be of a different species or genus from that inoculated in the first passage, it may be the *Plasmodium* of the latent infection which is transmitted. This would be most likely, if the inoculation be made into an animal of a species which had some considerable degree of tolerance to the former parasites and not to the latter.

It is easy to see how, during such passages, very great variations may appear to occur in the morphology and pathogenicity of what was thought to be a pure strain of one species of parasite. This would be the case especially when an infection is passaged through a long series of animals of different species or genera, in which the occurrence of latent or mixed infections has not been adequately excluded. At the end of such an experiment it is usually impossible to say, with any degree of certainty, that either the strain or species of *Plasmodium* which predominated in the first host is the same as that which predominates or is present in the last one. These numerous passages may have

added to or have eliminated various strains or species of parasite, so one cannot conclude that any variations in pathogenicity, shown by hosts of the same species at different stages of such experiments, are comparable (*vide infra* Leger and Bouilliez, 1913).

(d) CONCLUSIONS.

From the results of our experiments and the reports of other workers it seems probable that :—

(a) The pathogenic effects produced by a *Plasmodium* in different genera of monkey may vary, due to some natural immunity of the species.

(b) Variations in pathogenicity may occur on account of the presence of some degree of tolerance, acquired as the result of previous infection, but such tolerance may not be complete against superinfection with a heterologous strain of the same parasite.

(c) The degree of pathogenicity of different species of simian *Plasmodia* varies very markedly—(i) some species appear to produce mild infections in all the species of monkey tested, while (ii) others produce very severe infections in one species and not in another.

It has not been possible to determine whether the latter is due to a natural immunity of the species or to a tolerance acquired as the result of previous infection.

(d) The evidence that the virulence of strains of certain species of *Plasmodia* may be raised by repeated animal passage is not conclusive.

(e) Many of the reports of very varied pathogenicity, in what were considered to be the same strains and species of parasite, are probably due to the presence of undetected latent or mixed infections in some of the animals used for passage.

**D. ANALYSIS OF SOME OF THE EARLIER RECORDS, IN  
RELATION TO THE POSSIBILITY OF THE OCCURRENCE  
OF UNRECOGNIZED LATENT AND MIXED MALARIAL  
INFECTIONS.**

**I. THE RECORDS OF MAYER (1907, 1908).**

Mayer (1908) figures considerable differences in the size of the infested red cells in his infections. He also reports that he found great variations in the intensity of the stippling in such cells, and in the frequency of the occurrence of this phenomenon in experimentally infected animals. In some monkeys stippling was easily demonstrable even when a weak solution of Giemsa's stain was used, while in others it was much less marked even when a strong stain was employed. The alterations in the cells figured by Flu (1908), who used the same strain of *P. inui* var. *cynomolgi*, appear to be much more constant.

*Comment.*—While the morphology of the parasites figured by Flu (1908) suggests that he was dealing with a pure, or almost pure, infection with *P. inui*

var. *cynomolgi*, several of the figures given by Mayer (1908) suggest very strongly that he had a mixed infection with *P. knowlesi* in some animals. This view is also supported by the variability recorded in the occurrence of stippled red cells. This variability would appear to indicate that, while *P. inui* var. *cynomolgi* was the predominant parasite in most of the infections, yet some different species of parasite was present occasionally in some of the others.

## II. THE RECORDS OF GONDER AND BERENBERG-GOSSLER (1908), OF BERENBERG-GOSSLER (1909) AND OF GONDER AND RODENWALDT (1910).

(a) Gonder and Berenberg-Gossler (1908) and Berenberg-Gossler (1909) recorded natural malarial infections in *Cercocebus fuliginosus*. They have described in great detail the parasites found when young specimens of this monkey were inoculated from naturally infected animals. These parasites they believed to be identical with *P. kochi* (Lav.), as described by Kossel (1899). On the other hand, Sinton and Mulligan (1932) consider them to belong to a different species, for which they proposed the name *P. inui* var. *gonderi*.

The former workers state that in experimental infections observed in young specimens of *C. fuliginosus*, endoglobular forms with enlargement and stippling of the infested red cells occur, sometimes in great numbers. In natural infections, however, they had only seen the latter phenomenon in two red cells. They also report under these conditions, great variability in the amounts of pigment in the parasites, and the presence of certain forms with peculiar division and nuclear changes, which they think to be due to degeneration. Berenberg-Gossler (1909) concludes that monkey malarial parasites are very sensitive to changes in the natural condition of the body of the host.

*Comment.*—These findings suggest that a mixed infection was present in the natural host, in which one species of *Plasmodium* was almost exclusively predominant in the peripheral blood. This species of parasite caused no marked changes in the infested cells [? *P. kochi* (Lav.)]. When, however, the infection was transmitted by blood inoculation to other younger and more susceptible animals, the species of parasite which was latent became the predominant one (*P. inui* var. *gonderi*). This parasite produced marked cellular changes and had certain morphological differences from the former one. These results resemble the findings recorded by us with mixed infection of *P. knowlesi* and *P. inui* var. *cynomolgi*. The latter species of parasite predominates markedly in the natural host, while, when *S. rhesus* is inoculated, the former is the more common and often the only detectable parasite.

(b) Berenberg-Gossler (1909) and Gonder and Rodenwaldt (1910) record similar changes when animals with latent infections are splenectomized.

*Comment.*—This suggests that the specimens of *C. fuliginosus* used had some tolerance, either natural or acquired, to one of the species of parasite.

present in the primary infection, and that this tolerance to one species was more active than to the other. When a fresh animal was inoculated, the parasite against which the tolerance was less active predominated rapidly. A similar occurrence appeared to take place after splenectomy. This is very like the predominance of *P. inui* var. *cynomolgi* at the expense of *P. knowlesi* in artificial infections of *S. irus*, which species of monkey seems also to have a considerable tolerance to the latter parasite. Sinton and Mulligan (1933b) also found that while *P. inui* var. *cynomolgi* was usually the more conspicuous parasite in both natural and experimental mixed infections in *S. irus*, yet when the immunity of the latter animal was lowered by splenectomy, it was *P. knowlesi* which rapidly predominated. This result is very similar to that observed by Berenberg-Gossler (1909) and by Gonder and Rodenwaldt (1910). It adds considerable support to the suggestion that these workers were dealing with an unsuspected mixed infection.

(c) Berenberg-Gossler (1909) found that the fever in his inoculated animals was very irregular. He concluded that the cycle of schizogony was not of a regular type but varied in duration from 24 to 50 hours. On the other hand, Gonder and Rodenwaldt (1910) found that, when artificially infected animals with latent infections were splenectomized, numerous parasites reappeared in the peripheral blood, and the fever was typically tertian in character.

*Comment.*—This suggests either (i) that the fever in the acute artificial infections had not settled down to its tertian periodicity, a condition also recorded by James (1926) in acute experimental infections with *P. vivax*, or (ii) that there was a mixed infection during the acute attack, which was reflected in the temperature curves, while in the chronic infection the predominant parasite had a tertian periodicity.

### III. THE RECORDS OF MATHIS AND LEGER (1911).

(a) Mathis and Leger (1911) studied natural infections in *S. lasiotis tcheliensis* and *S. rhesus* in Tonkin. These infections they considered to be due to *P. inui*. They state that stippling of the infested blood cells was often seen when Leishman's stain was used, but at other times they failed to detect such changes without being able to determine exactly the cause of this failure. Attempts to demonstrate this phenomenon with Giemsa's stain were always unsuccessful, even when special techniques were tried.

*Comment.*—These workers studied the parasites in chronic infections. Their failure to find stippling constantly, suggests that some of these animals may have had very scanty infections with *P. inui* var. *cynomolgi*, or a similar parasite producing stippling, as well as with *P. inui* (sens. restr.).

As judged by the records of monkey infections in Malaya, the former species of parasite is not uncommon among the monkeys of the Orient. The occasional occurrence of *P. inui* var. *cynomolgi* or a similar species in the peripheral blood would afford an explanation of their findings.



## IV. THE RECORDS OF BLANCHARD AND LANGERON (1912, 1913).

(a) Blanchard and Langeron (1912, 1913) described a parasite in the blood of *S. irus* (*M. cynomolgus*) in Paris. They identified this parasite as *P. inui* var. *cynomolgi*. In their first paper these workers described the parasite as studied by them in a fatal infection in an inoculated specimen of *S. irus*. This animal had previously been under observation for about a year, and had shown no signs of malarial infection.

*Comment.*—Their figures suggest that in this animal both *P. knowlesi* and *P. inui* var. *cynomolgi* were present. This combination is very common in our experience of natural infections in *S. irus*.

(b) In their second paper, many of the illustrations are from chronic inoculation infections in *S. irus*, and from animals with similar infections which had been splenectomized. Blanchard and Langeron (1913) emphasize a change in the morphology of the parasite after splenectomy.

*Comment.*—Here the figures reproduced suggest that *P. inui* var. *cynomolgi* may have become the predominant parasite in the peripheral blood in these infections. Such a change is also supported by the statement that the number of merozoites increased, 16 being the common number found with this parasite. A predominance of this species of parasite is the usual finding in chronic mixed infections in this species of monkey. The splenectomy probably lighted this up, and also one of *P. knowlesi*, for both species of parasite seem to have been present in the acute infections. The lowered tolerance, following removal of the spleen, probably led to a more abundant appearance of *P. knowlesi* in the peripheral blood than is commonly seen in infections of *S. irus*. This has been our experience also after splenectomy in this species of monkey.

(c) The same workers (Blanchard and Langeron, 1912, 1913) report diverse clinical results when their infection was inoculated into other specimens of *S. irus*. Two of these animals died of very acute infections, while in six others the infection was of a more mild and chronic character.

*Comment.*—The former infections appear to have been much more severe than those recorded by any other workers who inoculated the same species of monkey with infections of *P. inui* var. *cynomolgi*. Mayer (1908), Flu (1908), Green (1932) and Sinton and Mulligan (1933a) all report comparatively mild infections following upon the injection of this parasite into *S. irus*. The last workers also record a mild infection in *S. rhesus*, an animal which is extremely susceptible to *P. knowlesi*, and which is probably not in a position to acquire any tolerance to malarial infections in nature in India. The descriptions given by Blanchard and Langeron (1912, 1913) of the fatal results resemble those seen when a mixed infection of *P. knowlesi* and *P. inui* var. *cynomolgi* is injected into a susceptible animal like *S. rhesus*. The mild infections are very similar to those observed by Knowles and Das Gupta (1932) and by Sinton and Mulligan (1933a), when such an infection is transmitted to a relatively immune host, such as freshly imported specimens of *S. irus*. It seems probable that the animals in which the acute infection occurred had either never acquired

any high degree of tolerance, or had lost most of this to the strain of *P. knowlesi* injected (*vide* Mulligan and Sinton, 1933). Such a possibility is strongly suggested by the history of the monkey, the study of whose blood is recorded in the first paper by Blanchard and Langeron (1912).

#### V. THE RECORDS OF LEGER AND BOUILLIEZ (1913).

Leger and Bouilliez (1913) report very diverse results, especially in regard to pathogenicity, when working with an infection diagnosed by them as due to *P. inui*. This infection was discovered as a natural infection in *S. irus*.

(a) The table and protocols given by these workers show 17 direct passages of the infection. During these it was passed through 8 specimens of *Silenus irus*, 4 of *S. sinicus*, and 1 each of *S. rhesus*, *S. nemistrinus*, *Cercopithecus callitrichus*, *C. cephus* and *Erythrocebus patas* (*C. patas*). In addition to these direct passages, infection was successfully conveyed at various times to 4 other specimens of *S. irus*, 2 of *S. rhesus*, 1 of *Papio anubis* and 1 of *E. patas*. Unsuccessful attempts were also made to infect 2 chimpanzees, 2 *S. irus*\*, a Lemur, a Hapale and a *Cercocebus fuliginosus* (3 times†).

Leger and Bouilliez (1913) state that 'un examen préalable des singes neufs a permis d'éliminer deux qui étaient déjà parasités'. The infected animals were one *Cercopithecus* and one *Silenus*.

*Comment.*—The results of the preliminary blood examination show that, including the original host, at least 3 of the lower monkeys used in these experiments had natural infections. These were of such a degree as to be detectable by a preliminary microscopical examination of the blood. The difficulty of detecting latent infections in experimental monkeys by this means has been discussed at some length by Sinton and Mulligan (1933b), and is borne out by the protocols given by Leger and Bouilliez (1913) (*vide* Monkeys Nos. 94 and 100, and Nos. 609 and 856). Under these circumstances, it appears impossible to exclude the chances that one or more of the 17 different animals, through which the infection was passaged, were not already suffering from an undetected latent infection before inoculation. This is especially the case when one considers the relative frequency of natural malarial infections in certain species of the genus *Silenus* and the genus *Cercopithecus* through which the infection was passaged (*vide* Appendix). This is apart from the chances that a latent mixed infection might have been present in some of the animals used.

These facts make it appear to us very unlikely that the strain, and probably even the species of parasite, which was studied in the primary natural infection, was the same which predominated or was present at the end of these numerous passages. Such passaging may have added new species or strains, or have

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\* Both these animals received injections of quinine at the same time as the first blood inoculations. A later attempt to infect one of them was successful, when quinine was not given.

† None of these unsuccessful attempts was made after the passage of the strain through the other African monkeys (*vide infra*).

eliminated some of these. This appears to us to be a very likely explanation of many of the varied results reported by Leger and Bouilliez (1913). Several of the other observations recorded by these authors would seem to support this idea.

(b) Leger and Bouilliez (1913) state that the parasites in the infections studied by them resembled in some respects each of the three common malaria parasites of man. Some points of resemblance in the case of *P. vivax* were its amoeboidicity, its 16 merozoites and the occurrence of stippling in the infested red cells. The latter phenomenon was said to be present only in certain animals and on certain days (*cf.* Mayer, 1908, *supra*). The presence of these cellular changes is first mentioned in the protocols after passage V, and the only references made to it are in connection with the protocols of *S. irus* (No. 99) and *C. cephus*. In both instances splenectomy had apparently been performed before the stippled cells were encountered, and the authors report that the stippling seen by them was most marked in a splenectomized animal. They also note in another portion of their paper that they were unable to find any stippling, even with Pappenheim's stain, in the later stages of their work.

*Comment.*—There are several points in the morphological and other characteristics of '*P. inui*', as described by these authors, which suggest that they were not dealing with the same species of *Plasmodium* in all their animals.

(i) The adolescent forms of the parasite are stated to be very amoeboid and rarely of a compact shape. This description differs considerably from the figures given by Halberstadter and Prowazek (1907) and by Mathis and Leger (1911), who previously described *P. inui* (*sens. restr.*). Unfortunately, no figures of the parasite have been given by Leger and Bouilliez, so ocular comparison is impossible. Nor have we been able to discover whether the description given of the parasite was founded on the examination of the blood of one animal, or represents a summary of the findings in several animals at different stages of the experiments.

(ii) In a preliminary paper, Leger and Bouilliez (1912) state definitely that the merozoites in the adult schizont numbered 16, when studied in *S. sinicus* (No. 94) infected at passage IV. On the other hand, Leger and Bouilliez (1913), while giving the number of merozoites as 16 in their parasite, state that there were only 8 in the large number of *mature* and *rupturing* schizonts seen at the death of *S. irus* (No. 819) after passage XI. The differences recorded were seen after the infection had been passed through 3 African monkeys.

(iii) The rarity of stippling suggests that an infection with *P. inui* var. *cynomolgi* might have been present in some animals, but that the number of parasites was only sufficient to make its presence conspicuous occasionally. The occurrence of stippling after splenectomy bears a striking resemblance to the results recorded by Blanchard and Langeron (1913) when working with *P. inui* var. *cynomolgi* (*vide supra*).

Another explanation might be that an infection with *P. knowlesi* was present in some cases. The red cells infested by the more advanced, growing

forms of this parasite show stippling, which is not always demonstrable by the usual staining with Giemsa's stain. It is, however, markedly shown by other methods, when the blood is taken at the proper stage of parasitic development. This would account for the occasional findings recorded by Leger and Bouilliez with Leishman's stain. As pointed out in this discussion, it also appears probable that several of the animals in these experiments were infected with different species of parasite, some of which might cause stippling in the infested cells.

The occurrence of stippling is only mentioned in the protocols after passage V, i.e., into *S. irus* (No. 99), and natural infections with both *P. knowlesi* and *P. inui* var. *cynomolgi* are not uncommon in this species of monkey. This suggests either (1) that one or both of these infections may have been introduced at passage IV from *S. irus*, No. 46, or (2), more probably, that they were present as latent infections in *S. irus*, No. 99. The latter possibility is supported by the record that this monkey failed to show infection at its first inoculation and only a mild one after the second. This would appear to indicate that some tolerance, possibly due to a latent infection, may have been present.

The very pleomorphic characteristics ascribed to the parasites, observed in these experiments, would be more in keeping with the presence of mixed infections, or the introduction of another species of parasite, rather than with a pure infection of *P. inui* (sens. restr.), as originally described by Halberstadter and Prowazek (1907).

The complete absence of demonstrable stippling in the later work, even when Pappenheim's panoptic stain was used, may indicate that an entirely new species of parasite had been introduced after passage through the African monkeys. The common African *Plasmodium* of the lower monkeys, *P. kochi* and its varieties, apparently cause no stippling of the infested red cells (*vide* Sinton and Mulligan, 1932). This suggestion is discussed more fully when the other data given by Leger and Bouilliez are analysed.

(c) The two specimens of *S. sinicus* used in the first two passages succumbed to very acute infections, while the two animals of this species used in passages IV and XVI developed mild attacks.

*Comment.*—This suggests that a change in the strain or species of parasite had occurred in the meantime. The number of animals infected is, however, small. Apart from these observations of Leger and Bouilliez, nothing seems to be known about the pathogenicity of *P. inui* (sens. restr.) in *S. sinicus*, while *P. knowlesi* usually produces a milder infection in this species than in *S. rhesus* (Knowles and Das Gupta, 1932; Sinton and Mulligan, 1933a).

(d) Seven specimens of *S. irus* were inoculated during the first eight passages (Nos. 46, 118, 1000, 99, 13, 41 and 332), and only 2 deaths were recorded. One of these (No. 1000) died with an acute malarial infection, while the second death (No. 332) was attributed to quinine poisoning. On the other hand, after the infection had been passaged through the 3 African monkeys (passages VII to IX), 5 out of 6 specimens of *S. irus* developed acute infections

and died (Nos. 306, 819, 818, 13 and 69). The only animal which survived (No. 847) had been given a prophylactic quinine injection at the same time as the blood inoculation.

*Comment.*—These findings suggest the possibility of the introduction of an African species of parasite, which caused a severe infection in the Asiatic monkey, *S. irus*. This infection in the African monkeys may have been the result of the reawakening of a latent infection caused by the injection of blood from an animal of a different genus (Berenberg-Gossler, 1909; Knowles, 1919). Unfortunately, there seem to be no records of the pathogenicity of any of the African Plasmodia in Oriental monkeys, apart from the unsuccessful attempt of Martoglio *et al.* (1910) to transmit *P. kochi* var. *joyeuxi* to *Silenus* sp.

It is interesting to note that Monkey No. 13 (*S. irus*), which proved refractory at passage VI, developed a very acute and fatal infection when re-inoculated at passage XIII. The former inoculation was made before the infection had been passed through any of the African monkeys, and the latter afterwards.

(e) Hæmaturia in *S. rhesus* (No. 98) and hæmoglobinuria in *C. callitrichus* are recorded as passages IV and IX respectively.

*Comment.*—These findings suggest that the same parasite may have been responsible for the two infections. *P. knowlesi* is the only parasite of monkeys which is certainly known to be capable of producing hæmoglobinuria\*, and this parasite is not uncommon as a latent, and usually unsuspected, natural infection in *S. irus*. It appears possible, therefore, that an infection with *P. knowlesi* may have been introduced at passages III or IV, where this species of monkey was used (Nos. 46 and 99).

*S. rhesus* (No. 100) was inoculated at passage V from the mild infection in *S. sinicus* (No. 94), which had been injected with the blood of *S. irus* (No. 46). The first animal had been given an injection of quinine when the parasites became numerous in the primary attack, but it died during a relapse 25 days after the original inoculation. The history of these two specimens of *S. rhesus* (Nos. 98 and 100) are very suggestive of an infection with *P. knowlesi*, transmitted from *S. irus* (No. 46) at passage IV.

(f) *C. callitrichus* inoculated at passage IX died with a very acute illness accompanied by hæmoglobinuria. The specimen of *S. irus* (No. 306) inoculated from this animal, and four other animals of the same species (Nos. 818, 819, 13 and 69) also died with very acute infections during subsequent passages.

*Comment.*—As mentioned above, the history of the infection in the African monkeys closely resembles that seen in susceptible animals infected with *P. knowlesi*. On the other hand, the inoculation of *S. irus* from *C. callitrichus* caused a severe and fatal infection. Our experience, and that of Knowles and Das Gupta (1932), has been that the former species of monkey is very resistant

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\*Sinton and Mulligan (1933b) report hæmoglobinuria in *S. rhesus* infected with *P. inui* var. *cynomolgi*. This occurrence was present only after the animal had been splenectomised.

to the pathogenic effects of both *P. knowlesi* and *P. inui* var. *cynomolgi*. This supports the idea that a foreign species or strain of parasite had been introduced at this stage through the African monkey, *C. callitrichus*. On the contrary, the results, recorded by Blanchard and Langeron (1912, 1913) and discussed above, would appear to indicate that *S. irus* is not always so immune to the pathogenic effects of *P. knowlesi*. The latter workers, however, found only 2 out of 9 monkeys so susceptible. It would, therefore, seem a remarkable coincidence, if Leger and Bouilliez (1913) should find 5 such animals in succession, non-immune or susceptible to severe infection with either *P. knowlesi* or *P. inui* var. *cynomolgi*.

The evidence available suggests very strongly that an African strain of parasite was introduced, which was highly pathogenic to some of the lower Oriental monkeys.

### Summary.

The very varied pathogenicity recorded by Leger and Bouilliez (1913), when '*P. inui*' was inoculated into a number of different species of monkey, may have been due partly to differences in the individual susceptibility or immunity of the different animals used. We consider, however, that such an explanation cannot be accepted in all instances, or should only be accepted with very considerable reservations. There is much internal evidence in their paper to suggest that these pathogenic results were due in many cases to the introduction of other strains or species of parasite, during the long series of passage experiments carried out by these workers.

The morphological features recorded also suggest that the infections produced during these passages were not all due to the same species of *Plasmodium*. An analysis of the evidence makes it appear possible that the infection after passages I, II and possibly III also may have been due to *P. inui* (sens. restr.). Those from passages IV to IX were probably due to *P. knowlesi*, or to a mixed infection of this parasite with *P. inui* var. *cynomolgi* in some cases. On the other hand, most if not all the infections after passage X were probably due to an African species of *Plasmodium*, or to a mixed infection of this with an Oriental one. The former parasite appeared to predominate and cause the pathogenic effects in most cases.

## VI. RECORDS OF OTHER WORKERS.

(a) Knowles and Das Gupta (1932) have described in great detail a malarial infection discovered in *S. irus*. They have recorded very marked changes in the morphology and pathogenicity of the parasites found, when the infection was passaged by blood inoculation to other Anthropeida of different genera and species from the original host.

Knowles (1932a) reports that with these parasites 'three factors appear to vary together and simultaneously; viz., (i) the amœboid or non-amœboid character of the parasite; (ii) the enlargement or non-enlargement of the red

corpuscle; and (iii) the presence or absence of stippling'. Great variations in pathogenicity are also reported. Although Knowles and Das Gupta (1932) suggested the possibility that these marked changes might be due to a mixed infection, they considered that this was not the case. A careful study of the data given by these workers has been made, and further experiments carried out with the same and similar strains of parasite by Sinton and Mulligan (1933b). The latter workers came to the conclusion that there could be little doubt but that the original infection studied by Knowles (1932a) and by Knowles and Das Gupta (1932) was an undetected mixed infection.

(b) In the past a large number of different blood pictures have been reported in natural malarial infections of the lower monkeys of Africa (*vide* Sinton and Mulligan, 1932). These infections have mostly been considered as due to '*P. kochi*'. No worker seems to have considered the possibility that some of the variations, recorded in the different parasites identified as '*P. kochi*', may have been due to mixed infections of which this species formed the basis. This aspect of the case requires careful consideration before the classification suggested by the latter workers can be accepted *in toto*. Sinton and Mulligan (1932) have separated the Plasmodia seen in these 'parasite pictures' of the blood of African monkeys into different varieties of *P. kochi*. This was an attempt to avoid further confusion in the already chaotic condition into which the classification of these parasites had fallen. It appears probable that the predominant parasite in the varieties erected by them is a true species or variety, but that the precise morphological picture of these Plasmodia has been obscured to some extent by a mixed infection with *P. kochi*, or some other species of *Plasmodium*. Much further experimental work is required to clear up these points.

(c) In the absence of more definite details, it is impossible to express any opinion upon the morphological changes, which Taliaferro (1932) has recorded in the parasites of the Panamanian monkeys.

### E. CONCLUSIONS.

There is very convincing evidence to suggest that :—

1. Natural mixed infections are not uncommon in the malaria of some of the common Oriental and African monkeys used for experimental purposes in laboratories.

2. Latent infections are also not rare among these lower monkeys, and are usually very difficult to exclude in experimental work. These, if undetected, may lead to artificially-produced mixed infections during the course of such work.

3. The occurrence of such mixed infection, either natural or experimental, has not been recognized in the past. This fact appears to have been responsible for many of the divergent reports published about the morphology and pathogenicity of the Plasmodia of the lower monkeys, at least of the Oriental species.

4. In many instances, the descriptions given by various workers of simian *Plasmodia*, identified as the same species, differ markedly in some essential details. The specific morphology of these parasites will require re-investigation in the light of the suggestions made in this paper.

5. The question, whether some of the other species of blood protozoa, which have been created upon differences in immunity rather than in morphology, are not due to unsuspected mixed infections, will require consideration.

*Postscript.* It will probably appear to the reader that in many places in the text of this paper an unnecessary amount of detail has been given. This has been intentional. Our aim has been that this paper, in combination with those of Sinton and Mulligan (1932, 1933a, 1933b), should include a full résumé of most of the important data given in previous reports about the malarial infections of the lower monkeys of the Old World. Much of this work is inaccessible, in its original form, to workers in the tropics. In view of the importance which this subject has attained inside the last few years, it appeared to us essential that such workers should have, easily available, some detailed information on previous work.

The authors also wish to apologise for the fact that the same data and arguments have been repeated in several places in this paper. This has also been intentional. We consider that, through the recognition of the possibility of mixed infections, a more satisfactory classification of the *Plasmodia* of the lower monkeys will eventually be reached. In order that the line of argument should be clear on each occasion, previous arguments have been repeated so that the reasons for our opinions should not be obscured.

## APPENDIX.

### RECORDS OF THE RELATIVE PREVALENCE OF NATURAL MALARIAL INFECTIONS AMONG THE LOWER MONKEYS.

Sinton and Mulligan (1933a) have given a comprehensive list of the different species of lower monkeys of the Old World in which natural infections with *Plasmodia* have been recorded. In most instances, however, they have not given any indication of the relative prevalence of such infections among the monkeys in different areas.

This subject is closely connected with the occurrence of mixed infections, more especially in relation to the possibility of the production of such infections during experimental work with these animals. Although numerous isolated instances of malarial infections in monkeys have been reported by various workers, in many cases no indication has been given as to the relative frequency of such infections in nature. We have listed below most of the records we have been able to find, in which some details of the degree of prevalence are noted.

### RECORDS OF THE RELATIVE PREVALENCE OF NATURAL MALARIAL INFECTIONS AMONG THE LOWER AFRICAN MONKEYS.

#### Genus *Colobus*.

Schwetz (1933c)\* reports from the Belgian Congo the examination of 2 specimens of this genus, both of which were infected.

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\* Records not given previously by Sinton and Mulligan (1932, 1933a).



Genus *Cercopithecus*.

Kossel (1899) found 15 out of 28 specimens of *C. sabaeus* from East Africa infected. Two specimens of the same species examined by Berenberg-Gossler (1909) at Hamburg were both infected, and Martoglio *et al.* (1910) records that 16 specimens of this animal from Ethiopia all showed Plasmodia.

Joyeux (1913) reported that 3 out of 9 specimens of *C. callitrichus* showed infections in French Guinea. Seidelin and Connal (1914) examined one specimen of *C. mona* in Nigeria and found parasites in its blood. Dutton, Todd and Tobey (1906)\* record 1 out of 40 specimens of *C. schmidtii* infected in the Congo Free State. Kinghorn and Yorke (1912) report malaria parasites as very common in the blood of specimens of *C. pygerythrus* in N. E. Rhodesia. Of 30 specimens of *C. callitrichus* and *Erythrocebus patas* examined in Central Africa by Bouilliez (1913), one of the former species was found infected. Theiler (1930) found parasites in each of 3 specimens of *C. diana* and 1 out of several specimens of *C. nictitans* examined by him in Liberia.

Among the specimens of *Cercopithecus* sp. used in experimental work on sleeping sickness in Uganda, Bruce and Nabarro (1903) and Bruce, Nabarro and Greig (1903) reported 7 (? 8) out of 10 animals infected with malaria. In other specimens of this genus, Gray and Tulloch (1907) found 9 out of 12 infected in the same area. Dutton, Todd and Tobey (1906) also record that these animals are frequently infected in the Congo Free State. Schwetz (1933c)\* states that 47 out of 60 specimens of this genus examined by him in the Belgian Congo were infected with malarial parasites.

Genus *Cercocebus*.

Berenberg-Gossler (1909) found that 3 out of 8 specimens of *C. fuliginosus* were infected at Hamburg, while Schwetz (1933c)\* reports that 15 out of 43 animals of this genus showed Plasmodia in the Belgian Congo.

Genus *Papio*.

Kossel (1899) reports 4 out of 21 specimens of this genus (? *P. babuinus*) from East Africa showed parasites in their blood. Seidelin and Connal (1914) also record that two specimens of *P. sphinx* examined by them in Nigeria were both infected. Lamborn (1929) examined 5 specimens of *P. pruinus* in Nyasaland, all of which were parasitized. In this genus, Schwetz (1933c)\* found one out of 5 specimens with malarial parasites in its blood in the Belgian Congo.

Genus *Callithrix*.

Cowdry and Cowell (1929) report that 2 out of 3 specimens of this genus ('closely resembling *C. personata*') received from Senegal were infected.†

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\* Records not given previously by Sinton and Mulligan (1932, 1933a).

† Some doubt exists as to the identity of these monkeys, as the genus *Callithrix* is indigenous in America and not in Africa (*vide* Sinton and Mulligan, 1932, 1933a).

*Miscellaneous.*

Duke (1921) states that malaria is very common among laboratory monkeys in Nyasaland. These monkeys were 'the white-nosed "nakabugo" and a black species with a somewhat baboon-like face'. Leger (1928) records that of 60 monkeys captured in Senegal and French Guinea, one *Papio* sp., one *Cercopithecus campbelli* and two *C. callitrichus* were found infected. Schwetz (1933a)\* examined 11 chimpanzees, 2 *Colobus*, 5 *Cynocephalus*, 60 *Cercopithecus* (of several species) and 43 *Cercocebus* (of 2 species) and reports that 60 per cent of these animals in the Belgian Congo were found infected.

These records indicate that the natural occurrence of malarial infections among the lower monkeys of Africa, more especially in the genera *Cercopithecus* and *Cercocebus*, are not uncommon in some localities.

RECORDS OF THE RELATIVE PREVALENCE OF NATURAL MALARIAL  
INFECTIONS AMONG THE LOWER ASIATIC MONKEYS.

*Genus Pygathrix.*

The record of Knowles (1919) from Assam of a malarial infection in *P. entellus* appears to be the only certain record of such an occurrence in this genus. Donovan (1920) reports that he found no infection in 20 specimens of *P. priamus* from South India.

*Genus Silenus (Macacus).*

Bruce and Nabarro (1903) record 1 infected animal out of 6 specimens of 'pale-faced' monkeys in their laboratory in Uganda (experiment 56, p. 31). These 'pale-faced' monkeys are later described by Bruce, Nabarro and Greig (1903) as *S. rhesus*, but in the latter report the solitary infected monkey of this type is recorded as *Cercopithecus* sp. (experiment 56, p. 33). These statements make it very doubtful whether one should consider this record of a natural infection in *S. rhesus* as valid. Even if it be valid, the chances of this Indian monkey having acquired a local infection from the numerous infected specimens of *Cercopithecus* sp. reported at the same time and place, must be considered.†

Mathis and Leger (1911) examined 40 specimens of *S. rhesus* and *S. lasiotis tcheliensis* in Tonkin and found that 5 of these showed Plasmodia in their bloods. Unfortunately these workers do not record the specific details of their findings. Knowles and Das Gupta (1932) state that in Calcutta they 'examined many batches of monkeys—chiefly *Macacus rhesus*—but previously with negative results'. The authors of this paper have now examined over 300 specimens of *S. rhesus* from northern India and have failed to find a single naturally infected animal. Kossel (1899) states that he found no infections among 6 monkeys from India examined by him.

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\* Records not given previously by Sinton and Mulligan (1932, 1933a).

† Duke (1921), also working in Uganda, thought that some of his laboratory monkeys became infected under very similar conditions.

Donovan (1920) examined the blood of 76 specimens of *S. sinicus* from Southern India and found none of them infected. He notes, however, that he afterwards received a blood slide from an animal of this species which showed parasites.

Mayer (1908) examined 4 specimens of *S. irus* (*M. cynomolgus*) recently imported from Java, and found them all infected. Green (1932) records 3 out of 12 specimens of the same species infected in Malaya. In this laboratory we have found infections in 6 out of 31 monkeys identified for us as *S. irus* and said to have come from Singapore.\*

These records suggest that in Malaya, Java and possibly further east, the occurrence of natural malarial infections among monkeys is not uncommon. On the other hand, such infections would appear to be rare in India. In spite of the very numerous examinations made of the common brown monkey of northern India, *S. rhesus*, no natural infections appear to have been recorded in this country. The only records of natural infections in this animal are (a) that of Chimisso (1922) in a specimen seen in Italy and said to have come from India, (b) the report of Mathis and Leger (1911) from Tonkin, and (c) the very doubtful record of Bruce and Nabarro (1903) from Uganda.

Except for the records of *P. semnopithecii* by Knowles (1919) in *Py. entellus*, of the same species of parasite by Wenyon (1926) in *S. pileatus* (?), and that of *P. inui* (?) by Donovan (1920) in *S. sinicus*, no natural infections appear to have been recorded in India. When one considers the large number of animals which have been examined, one seems safe in assuming that such natural infections must be of extreme rarity, at least in Northern India if not in other parts of this country.

This state of affairs makes the conditions for research into the morphology and pathogenic effects of monkey malaria parasites especially favourable in India. Once a strain of parasites has been established, the presence of large numbers of easily obtainable monkeys, which are uninfected and highly susceptible, greatly facilitates the work.

#### RECORDS OF THE RELATIVE PREVALENCE OF NATURAL MALARIAL INFECTIONS AMONG THE LOWER MONKEYS OF THE NEW WORLD.

The only worker who seems to have made a systematic study of this subject is Clark (1930, 1931). This worker examined smears of the blood and internal organs of a large number of monkeys from the Panama region. The majority of these observations were made on animals shot in localities far removed from human habitations.

\* Four different consignments of young specimens of *S. irus* have been obtained by us from Calcutta during the past year. In the first lot 3 animals were infected out of 5 received. One animal was found infected in each of the other three batches, which consisted of 6, 12, and 8 animals respectively.

Clark (1930) gives the following records in his first report :—

- (a) *Ateles geoffroyi* Kuhl (the red spider monkey)—7 specimens infected out of 24 examined;
- (b) *Cebus capucinus imitator* (Linnaeus) (the white-throated capuchin)—3 infected out of 31 animals;
- (c) *Saimiri orstedii orstedii* (Reinhardt) (the Chiriqui titi monkey)—none infected among 54; and
- (d) *Alouatta palliata inconsonans* Goldman (the Panama howling monkey)—none infected among 4 specimens.

In the second investigation by Clark (1931), the following additional records were obtained :—

- (a) *At. geoffroyi*—34 infected out of 75 animals;
- (b) *C. c. imitator* and *C. c. capucinus*—9 infected out of 55;
- (c) *S. o. orstedii*—none infected among 60 animals;
- (d) *Al. p. inconsonans*—2 out of 8 animals infected;
- (e) *Aotus zonalis* Goldman (the night monkey)—none infected among 4 examined, and
- (f) *Leontocebus geoffroyi* (Pucheran) (the squirrel monkey-mono titi)—none infected among 50 animals examined.

The positive records in Clark's work may be summarized as follows :—

- (a) *At. geoffroyi*—41 infected out of 99 specimens (40 per cent);
- (b) *C. c. imitator* and *C. c. capucinus*—12 infected out of 86 specimens (14 per cent);
- (c) *Al. p. inconsonans*—2 infected out of 12 animals (17 per cent);
- (d) Total—55 out of 197 animals examined of these three species were found infected, i.e. about 27 per cent.

Clark (1930) noted that infections were most common in infant and juvenile specimens, and among adult females which were pregnant. In his second paper (Clark, 1931), he records in the three species mentioned above that 9 out of 22 infants (41 per cent), 23 out of 25 juveniles (92 per cent) and 13 out of 91 adults (14 per cent) were infected, or a total of about 32 per cent of animals.

These records indicate that malarial infections in these species, especially among young animals, are very common in the localities where Clark made his collections.

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## STUDIES IN IMMUNITY IN MALARIA.

### Part III.

#### *MULTIPLE SUPERINFECTIONS WITH VARIOUS STRAINS OF PLASMODIUM KNOWLESI.*

BY

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### INTRODUCTION.

THE results of single superinfections with either homologous or heterologous strains of monkey malaria parasites were given in a previous paper (Mulligan and Sinton, 1933).

Some of the conclusions reached as the result of these investigations were that, under the conditions of the experiments :—

- ' (1) Several strains of *P. knowlesi* with different immunological characters have been isolated from the Malayan monkey, *S. irus*.
- (2) A chronic or latent infection with one strain of *P. knowlesi*, or with one strain of *P. inui* var. *cynomolgi*, appears to confer an effective immunity against the clinical effects of superinfection with the same strain of parasite.
- (3) A chronic or latent infection with one strain of *P. knowlesi* does not confer any effective immunity against the occurrence of an acute attack of malarial fever, produced by a superinfection with a different strain of the same parasite.



- (4) A chronic or latent infection with one strain of *P. knowlesi* appears to confer some tolerance to the clinical effects of superinfection with a different strain of the same parasite. This is indicated by an increased tendency for the initial acute attack of the superinfection to recover spontaneously, and by a diminished tendency for such infections to relapse at a later date.
- (5) The immunity produced by a given strain of parasites appears to be mainly specific for that strain. There is, however, some evidence to suggest that a slight degree of common immunity exists between strains of the same species of parasite. This is possibly non-specific and due to a general stimulation of the reticulo-endothelial system by such malarial infections'

Since the latter results were published, further investigations have been made to determine whether superinfection of the same monkeys with several heterologous strains of *P. knowlesi* would produce any greater degree of tolerance to superinfection with a strain which had not been tried previously. If it were found that superinfection with several different strains of the same species of *Plasmodium* produced a high degree of tolerance to *all* other strains, it would have an important bearing upon the question of the use of 'salted labour' in industrial undertakings. If, on the other hand, it were found that no considerable degree of enhanced tolerance developed, it would suggest that such labour was mainly 'salted' only against the strains of parasite prevalent in the locality from which these men were recruited.

#### STRAINS OF *P. KNOWLESI* USED.

The experimental work recorded in this paper was done with strains of *P. knowlesi* Sinton and Mulligan, 1932. These strains were isolated from seven different specimens of *Silenus irus*\* (*Macacus cynomolgus*, *Macacus fascicularis*), purchased in Calcutta and said to have been imported from Singapore.

Seven 'strains' of this parasite were available for these investigations:—

(a) Strain 'C' was originally discovered by Napier and Campbell (1932) and was the subject of considerable study by Knowles and Das Gupta (1932).

(b) Strains 'K<sub>1</sub>', 'K<sub>2</sub>' and 'K<sub>3</sub>' were isolated by us from three infected monkeys found in a batch of five specimens obtained in August 1932.

(c) Strain 'K<sub>4</sub>' occurred in one of a lot of six monkeys received in December 1932.

(d) Strain 'K<sub>5</sub>' occurred in a monkey acquired in March 1933 with 11 other monkeys.

(e) Strain 'K<sub>6</sub>' was discovered in one of a batch of eight monkeys purchased in May 1933.

\*This species of monkey, the crab-eating macaque, is indigenous in Burma, Siam and the Malay Peninsula.

All the monkeys mentioned were of the same species (*S. irus*). The infections from which these 'strains' were isolated were in nearly every instance mixed ones of *P. knowlesi* and *P. cynomolgi*. The methods used to obtain pure infections of each species of *Plasmodium* have been described by Sinton and Mulligan (1933).

When these parasites were first isolated from different specimens of *S. irus*, they were called 'strains' for ease of reference. There was no conclusive evidence at that time that these 'strains' were immunologically different, nor that any one was not a combination of several different ones with varied immunological characters. It appeared possible that strains K<sub>1</sub>, K<sub>2</sub> and K<sub>3</sub> might be of this nature, because they were all isolated from the same group of monkeys. The work on superinfection with these strains (Mulligan and Sinton, 1933) and later investigations, suggest that the latter strains are not identical in all respects, but may be composed of several immunological 'strains' in varying combinations.

#### METHODS, TECHNIQUE, ETC.

The methods used in our superinfection experiments have been described in some detail by Mulligan and Sinton (1933). Similar methods were employed in the present investigations.

##### (a) Inoculation.

It must be remembered, in drawing any practical deductions from the results of our experiments, that the transmission of the infection was made in all cases by blood inoculation, and not by mosquito bite as in nature. The work on therapeutic malaria has shown that, in respect to treatment at least, infections produced by these two methods may vary considerably.

Our results in simian malaria after superinfection by blood inoculation, have been very similar to those obtained by this method in human cases. The results obtained by James and others by mosquito transmission have shown little difference from those recorded by other workers with the former method. Under these conditions one appears to be justified in drawing some tentative conclusions from the results obtained in our work.

##### (b) Intervals between inoculations.

The work of superinfecting the same animal with several heterologous strains of *P. knowlesi* takes a long time. A sufficient period must be allowed to elapse between each superinfection, to give the infected host ample opportunity to develop a considerable degree of tolerance to each strain used. It is also necessary to allow for the possible occurrence of a prolonged incubation period in any one case\*, before the effects of another superinfection are tested.

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\* *Vide* experiments (n) and (o) of Mulligan and Sinton (1933).

It will be noted that in several instances recorded in this paper, especially with homologous superinfections, the intervals between some of our experiments were of much shorter duration than in our first series (Mulligan and Sinton, 1933). It was thought that the reticulo-endothelial system would be so stimulated and 'tuned-up' by the prolonged chronic infections, and the previous repeated superinfections, that tolerance would be more quickly developed than in the case of primary infections. This appeared to be the case when strains  $K_1$ ,  $K_2$ , and  $K_3$  were used.

The results obtained with strain  $K_4$  suggest that in some cases a longer interval may be required. The latter strain has been found to be the most virulent of the strains used by us, and shows a tendency to severe relapses, in many cases for long periods after the primary acute attack. These relapses may be so severe as to require treatment. Great difficulty was also experienced in establishing chronic infections with this strain, as many of the animals died during relapses. These results indicate that with strain  $K_4$  a much longer period is required for the development of a high degree of tolerance. It will be noted that the last superinfection in experiment (vii) (*vide infra*) was made with strain  $K_6$  about 40 days after the previous one with strain  $K_4$ . The result of the former superinfection ( $K_6$ ) proved fatal. There is some reason to suspect that this superinfection may have been superimposed upon a developing relapse of strain  $K_4$ , and the fatal result may have been due to a combination of the two strains.

The apparent more rapid development of tolerance with strains  $K_1$ ,  $K_2$ , and  $K_3$ , when used for superinfections, may perhaps be attributable to some common factor in these strains. It, therefore, appears that a longer period than six weeks should be allowed to elapse between superinfections, more especially if the strain being investigated is unrelated to the others used.

Deaths due to the severity of heterologous superinfections have in several instances reduced the number of our experimental animals. These factors, as well as the large amount of routine work involved in the experiments, have necessarily curtailed the numbers in the series of multiple superinfections recorded in this paper.

### **(c) Effects of treatment on the development of tolerance.**

James (1931) and Cuica (1931) report that treatment of the acute attack in benign tertian malaria tends to diminish the development of tolerance. On the other hand, the cases of malignant tertian malaria recorded by James, Nicol and Shute (1932) appeared to have developed a considerable degree of tolerance, in spite of the fact that quinine had to be used to control the acute attacks.

It will be seen that, except in the primary infections and in primary heterologous superinfections, few or none of our animals received any treatment, apart from an occasional dose of stovarsol to animals with severe anæmia. The amount of treatment given in any attack was the minimum which appeared

to us to be compatible with saving the life of the animal. Indeed, in some cases the amounts were cut so low that the animals died.

As many of our animals had severe relapses in which no treatment was given, and as the duration of the chronic infection was very prolonged in many cases, it did not appear to us that the amount of treatment given interfered seriously with the development of tolerance. The fact that the results of homologous superinfection were mild or negligible, also indicates that the more prolonged treatment given in the primary infection had not a very marked effect in preventing the acquisition of a high degree of tolerance to the infection present.

#### (d) Evaluation of the effects of superinfection.

The results of superinfection upon the health and the parasite picture of the infected animals have been evaluated in a manner practically the same as that used in the previous experiments (Mulligan and Sinton, 1933). In those investigations

'The term "relapse" has been used to denote an increase or recrudescence of parasites in such numbers as to cause a distinct disturbance of the health of the animal. This may be shown by fever or some obvious signs of illness or malaise. The periodical reappearance of parasites in scanty numbers, without any apparent effect on the health of the animal, is not considered to be a relapse in the sense employed in these experiments. Such a reappearance can often be detected only by daily blood examinations and, in the absence of these examinations, such increases are likely to be missed'.

The 'clinical relapses' referred to above are a prominent feature of most primary infections, and many of the primary superinfections with heterologous strains. While such relapses are very convenient for evaluating the results of the infections mentioned, the clinical manifestations in the multiple superinfections were usually much less marked. Under the latter conditions, it has been found necessary to judge the results mainly by changes in the intensity of the parasitic infection. While many of these parasitic relapses would probably be severe enough to produce complaints in a human host, they were not sufficient to cause detectable symptoms in animals. Such relapses would therefore be missed if frequent, usually daily, blood examinations were not undertaken.

### SECTION I.

#### EFFECTS OF MULTIPLE INFECTIONS OF HOMOLOGOUS STRAINS OF *P. KNOWLESI* ON TOLERANCE TO SUPERINFECTION.

Four specimens of *S. rhesus* were each superinfected on several separate occasions with blood obtained from other monkeys infected experimentally with the same strain of *Plasmodium*. After a suitable period had elapsed these animals were again superinfected with the same strain, either from the original host of the strain (*S. irus*), or, if this was not available, with the same strain

TABLE  
Summary of protocols of

Experiment No.	Number and species of monkey. ( <i>Silenus</i> sp.)	PRIMARY INFECTION.			FIRST SUPER-INFECTION.				SECOND SUPER-INFECTION.			
		Strain.	Passage.	Result.	Days after 1st inoculation.	Strain.	Passage.	Result.	Days after 1st inoculation.	Strain.	Passage.	Result.
(i)	66 ( <i>rhesus</i> )	K <sub>1</sub>	2	+++ (RT)	209	K <sub>1</sub>	6	—	272	K <sub>1</sub>	8	—
(ii)	64 ( <i>rhesus</i> )	K <sub>2</sub>	OH	+++ (RT)	212	K <sub>2</sub>	2	±	251	K <sub>2</sub>	OH	+
(iii)	141 ( <i>rhesus</i> )†	K <sub>2</sub>	3	+++ (RT)	64	K <sub>2</sub>	1-3-1	—	133	K <sub>2</sub>	OH	+
(iv)	22 ( <i>rhesus</i> )	C	6a	+++ (RT)	458	C	7a	±	471	C	6a	—
(v)	7 ( <i>rhesus</i> )	C	1a	++ (RT)	343	C	4a	—	379	K <sub>1</sub>	6	+++ (RT)
(vi)	29 ( <i>rhesus</i> )	C	9a	+++ (RT)	258	C	5a	±	309	K <sub>1</sub>	6	+++ (RT)
(vii)	9 ( <i>rhesus</i> )	C	1a	+++ (RT)	343	K <sub>1</sub>	5	++ (SR)	379	K <sub>2</sub>	2	+
(viii)	39 ( <i>rhesus</i> )	C	2a	+++ (RT)	203	K <sub>2</sub>	1-3	+++ (RT)	287	K <sub>1</sub>	2	+
(ix)	80 ( <i>rhesus</i> )†	K <sub>2</sub>	OH	+++ (RT)	126	Cy	2	+++ (SR)	207	K <sub>1</sub>	2	++ (RT)
(x)	82 ( <i>siniacus</i> )	K <sub>2</sub>	2	+++ (RT)	173	K <sub>1</sub>	6	++ (SR)	246	K <sub>2</sub>	2-3	±

**Explanatory Note.** (a) Result:— — indicates no attack or appreciable increase in the number of parasites.  
 ± indicates a slight transient increase in the number of parasites.  
 + indicates a definite increase of parasites, without the production of clinical symptoms.  
 ++ indicates an attack of moderate severity.  
 +++ indicates a very severe attack.

## I.

*superinfection experiments.*

THIRD SUPER-INFECTION.				FOURTH SUPER-INFECTION.				FIFTH SUPER-INFECTION.				TOTAL.	
Days after 1st inoculation.	Strain.	Passage.	Result.	Days after 1st inoculation.	Strain.	Passage.	Result.	Days after 1st inoculation.	Strain.	Passage.	Result.	No. of homologous superinfections.	No. of heterologous superinfections.
292	K <sub>1</sub>	8	—	326	K <sub>1</sub>	2	+	361	C	7a	++ (SR)	4	1
295	K <sub>2</sub>	3	—	329	K <sub>2</sub>	OH	+	364	C	7a	+++ (D)	4	1
..	..	..	..	..	..	..	..	..	..	..	..	2	0
490	C	5a	—	514	C	8a	— (?)	..	..	..	..	4	0
427	K <sub>3</sub>	1-3	+	494	K <sub>3</sub>	1	—	523	K <sub>4</sub>	2-3	+	1	4
357	K <sub>3</sub>	1-3	±	423	K <sub>3</sub>	1	+	452	K <sub>4</sub>	2-3	+	1	4
427	K <sub>1</sub>	OH	±	484	K <sub>4</sub>	2-3	+	523	K <sub>6</sub>	OH	+++ (D)	0	5
344	K <sub>4</sub>	2-3	+	384	K <sub>5</sub>	OH	±	..	..	..	..	0	4
274	C	6a	—	305	K <sub>5</sub>	2	±	..	..	..	..	0	3
296	K <sub>6</sub>	OH	+	..	..	..	..	..	..	..	..	0	3

SR means spontaneous recovery.

RT means recovery following treatment.

D means death.

(b) **Passage:**—These numbers indicate the number of passages in series from the original host to the animal from which the inoculation was made. OH denotes the original host of the strain.

from *S. rhesus*. These experiments serve as controls for comparison with the results of superinfection with heterologous strains. It was also thought that these experiments might give some indication as to whether any change in the virulence or immunizing power of the strain had occurred as the result of repeated passage. The results are summarized in Table I.

(1) STRAIN 'K'.

(i) Monkey No. 66 (*S. rhesus*).\*

*History of primary infection.* Infected with strain K<sub>1</sub> from Monkey No. 61 (*S. rhesus*) (passage 2).† Blood taken during course of the acute primary attack. Parasites detected on 5th day and increased rapidly to reach maximum on 8th day: severe attack with pernicious symptoms resulted; quinine treatment given daily from 5th to 9th days, and from 11th to 13th days, arrested attack; relapse occurred on 19th day and again on 23rd day, but recovery followed a single dose of quinine on each occasion; further relapses requiring quinine treatment occurred on 26th, 41st, 48th and 67th days. Eventually infection became chronic and clinical relapses ceased; parasites observed on and off up to 200th day.

*Result.* A very acute infection with many clinical relapses requiring treatment.

*History of 1st superinfection.* Superinfected on 209th day with same strain. Blood taken from Monkey No. 99 (*S. rhesus*) (passage 6) during sub-acute stage of decline of primary attack. No marked change observed in parasitic findings after superinfection, although daily examinations made up to 251st day; slight transient increase in number of parasites noted on 220th day. Weekly examinations made later up to 272nd day only showed parasites on two occasions.

*Result.* No appreciable change in infection detected.

*History of 2nd superinfection.* Superinfected on 272nd day with same strain. Blood taken from Monkey No. 153 (*S. rhesus*) (passage 8) during acute primary attack. Very few parasites found on 273rd, 275th and 279th days, otherwise negative at daily examinations till 292nd day.

*Result.* No change in infection, although superinfected from animal in acute attack.

*History of 3rd superinfection.* Superinfected on 292nd day with same strain. Blood taken from Monkey No. 153 (*S. rhesus*) (passage 8) during chronic stage of infection. Daily blood examinations up to 326th day negative, except for very few parasites on 305th and 306th days.

*Result.* No appreciable change in infection.

*History of 4th superinfection.* Superinfected on 326th day with same strain of parasite. Blood taken from a chronic infection in *S. irus* (No. 55) (passage 2). Parasites detected on 340th day; this was followed by a definite increase in parasites reaching maximum on 351st day. At this time the animal became very anæmic, and stovarsol (1 grain) was given daily for 6 days. Parasites gradually diminished and not detectable from 356th to 361st days.

*Result.* A distinct parasitic relapse, with marked anæmia requiring treatment.

\*This animal is that used in experiment (d) by Mulligan and Sinton (1933).

†This indicates the number of passages in series, which had been made previously from the original infection.

**History of 5th superinfection.** Superinfected on 361st day with strain C. Blood taken from Monkey No. 167 (*S. rhesus*) (passage 7a)\* during chronic infection. Parasites again detected on 382nd day, increased rapidly causing an acute attack with clinical symptoms from 385th to 392nd day; recovery spontaneous. Parasites then gradually diminished and disappeared on 402nd day; not again detected up to 416th day.

**Result.** Superinfection with a heterologous strain caused a severe attack, which recovered spontaneously [cf. experiment (ii)].

### Results of multiple superinfections with strain K<sub>1</sub>.

It will be noted that three superinfections with the same strain of *P. knowlesi* between 209th and 292nd days produced no definite change in the infection. A fourth superinfection with the same strain on 326th day caused, however, a definite increase in the number of parasites.

The first three superinfections were made from *S. rhesus* after the strain had undergone 6-8 passages, while the fourth one was made, as in the primary infection, from the 2nd passage in *S. irus*. This result suggests either that there has been some diminution in the virulence of the strain during the numerous passages in *S. rhesus*, or that strains in *S. irus* retain a higher degree of virulence than in the former species.

The results of homologous superinfection appeared to be independent of whether the animal was superinfected from an acute or a chronic infection.

In spite of the marked tolerance produced by 4 superinfections with one strain of parasite, a 5th superinfection with a heterologous strain caused an acute attack with clinical symptoms. The incubation period of the latter infection was, however, prolonged.

### (2) STRAIN 'K<sub>1</sub>'.

#### (ii) Monkey No. 64 (*S. rhesus*).†

**History of primary infection.** Infected from Monkey No. 54 (*S. irus*) the original host of strain K<sub>1</sub>. Parasites detected on 8th day, but disappeared after quinine treatment given on same day; parasites reappeared on 14th day and increased rapidly, reaching maximum on 18th day; very acute attack which recovered with quinine treatment given on 16th, 17th and 19th days; two relapses requiring quinine treatment occurred on 24th day (2 days' treatment) and on 79th day (3 days' treatment); subsequently mild relapses occurred with spontaneous recovery; infection gradually became chronic with periodical appearance of scanty parasites up to 211th day.

**Result.** A very severe attack, with many relapses requiring quinine treatment.

**History of 1st superinfection.** Superinfected on 212th day with homologous strain. Blood taken from Monkey No. 98 (*S. rhesus*) (passage 2) during decline of

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\* The passages of strain C indicate, in all instances, the number of passages in series from the infected animal so kindly supplied to us by Colonel Knowles. We have no knowledge of how far removed this passage was from the original host.

† This animal is that used in experiment (e) by Mulligan and Sinton (1933).



acute primary infection. Slight transient increase in number of parasites from 233rd to 239th days, with slight anæmia requiring stovarsol; later parasites remained scanty or absent at daily examinations up to 250th day.

*Result.* No very marked change in infection, but slight parasite relapse.

*History of 2nd superinfection.* Superinfected on 251st day with same strain of parasite. Blood taken from Monkey No. 54 (*S. irus*) (original host). A very few parasites detectable at examinations made immediately after superinfection; these began to increase considerably and a moderate parasitic attack occurred with maximum about 269th day; after this the parasites gradually decreased and few or none were found at daily examinations from 279th to 295th days.

*Result.* A definite parasitic relapse without symptoms.

*History of 3rd superinfection.* Superinfected on 295th day with same strain. Blood taken from chronic infection in Monkey No. 141 (*S. rhesus*) (passage 3). Daily blood examinations showed very scanty parasites up to 303rd day and from 313th to 323rd days, otherwise negative.

*Result.* No appreciable change in infection.

*History of 4th superinfection.* Superinfected on 329th day with same strain. Blood taken from Monkey No. 54 (*S. irus*) (original host). No parasites detected till 336th day; these increased gradually and were moderately abundant from 358th to 362nd days.

*Result.* A definite parasitic relapse without symptoms.

*History of 5th superinfection.* Superinfected on 364th day with strain C. Blood taken from Monkey No. 167 (*S. rhesus*) (passage 7a) during a chronic infection. Very scanty parasites detected on 370th day and rapidly increased; very numerous on 376th day accompanied by clinical symptoms; animal died during night in spite of treatment (quinine and plasmoquine).

*Result.* A very acute infection proving fatal in spite of treatment [cf. experiment (a)].

### (iii) Monkey No. 141 (*S. rhesus*).

*History of primary infection.* Infected with strain K<sub>1</sub> from Monkey No. 98 (*S. rhesus*) (passage 3). Blood taken during chronic infection. Parasites detected on 7th day and quinine given same day; parasites increased rapidly and plasmoquine given on 11th day, which caused slight decrease during next 2 days; numerous again on 15th day and plasmoquine repeated; parasites remained numerous and quinine given from 17th to 21st days; animal aborted on 20th day; parasites remained absent or scanty from 22nd to 28th days, then again rose from 29th to 41st days, requiring quinine on 30th day. Anæmia marked on 42nd day and stovarsol given 43rd, 44th, 45th and 48th days; parasites present in scanty numbers almost daily till 65th day.

*Result.* A very acute infection followed by relapses and severe anæmia requiring treatment.

*History of 1st superinfection.* Superinfected on 65th day with same strain of parasite. Blood taken from chronic superinfection in Monkey No. 64 (*S. rhesus*) (passages 1, 3, and 1) [vide experiment (i)]. Parasites present in scanty numbers at time of inoculation and continued till 68th day, then few daily till 96th day; absent, except twice, up to 114th day; later few almost daily till 134th day.

*Result.* No apparent change in infection.

*History of 2nd superinfection.* Superinfected on 134th day with same strain. Blood taken from Monkey No. 54 (*S. irus*) (original host). Very few parasites on 135th and 136th days; absent from 137th to 140th days; marked increase on 141st and 142nd days, then decrease again; later only present in scanty or very scanty

numbers at most daily examinations up to 168th day; afterwards absent up to 190th day, except for one day.

*Result.* A distinct parasitic relapse without clinical symptoms.

### Results of multiple superinfections with strain K<sub>2</sub>.

*Experiment (ii).*—The four homologous superinfections made in this case caused no noticeable clinical effects, although in some instances there was a definite rise in the number of parasites. It is noteworthy that, as in experiment (i), the two superinfections made from *S. irus* caused more marked parasitic relapses than those made from *S. rhesus*, although the latter were made as early as the 2nd and 3rd passages. This effect was also seen in the 4th superinfection, when one would have expected a high degree of tolerance to the homologous strain and no appreciable effect from homologous superinfection.

The effects of a 5th superinfection, a heterologous one, proved fatal. This indicates that multiple homologous superinfections do not protect against the severe clinical effects of a heterologous superinfection in all cases [cf. experiment (i)].

*Experiment (iii).*—The primary superinfection produced no appreciable effects even although the donor monkey (*S. rhesus*) was infected with a first passage strain and had been superinfected with both 1st and 3rd passage strains. This finding does not support the suggestion that any apparent loss of virulence is purely the result of multiplicity of passages in *S. rhesus*. The effect of a later superinfection from the original host (*S. irus*) was to cause a distinct parasitic relapse due to *P. knowlesi*. This finding supports the view that the strain in *S. irus* is more virulent than the one in *S. rhesus*.

### (3) STRAIN 'C'.

#### (iv) Monkey No. 22 (*S. rhesus*).

*History of primary infection.* Infected with strain C from Monkey No. 20 (*S. rhesus*) (passage 6a). Blood taken during acute attack. Parasites detected 2nd day and rapidly increased; severe attack requiring quinine on 4th, 5th and 6th days and again on 13th, 16th and 17th; after which blood negative for few days, then scanty parasites from 21st to 31st days. Relapse requiring plasmoquine (1 day) and quinine (2 days) occurred from 33rd to 35th days; afterwards parasites on and off at daily examinations up to 121st day; weekly examinations later showed no parasites up to 464th day, although protein shock produced by intravenous injection of human blood on 437th day.

*Result.* A very severe attack with a severe relapse requiring treatment. No parasites seen for nearly one year afterwards.

*History of 1st superinfection.* Superinfected on 458th day with same strain. Blood taken from Monkey No. 167 (*S. rhesus*) (passage 7a) during chronic infection. Very slight rise of parasites on 464th to 466th days, afterwards absent up to 471st day.

*Result.* Only a slight and very transient appearance of parasites.

*History of 2nd superinfection.* Superinfected on 471st day with same strain. Blood taken from Monkey No. 103 (*S. irus*) (passage 6a) during a very acute and fatal

attack produced by splenectomy in the course of a chronic infection. No parasites detected at daily examinations up to 490th day.

*Result.* No change in infection observed. The donor animal was, however, being treated (quinine and plasmoquine) when the blood was taken.

*History of 3rd superinfection.* Superinfected on 490th day with same strain. Blood taken from Monkey No. 100 (*S. rhesus*) (passage 5a) during acute attack following splenectomy in chronic infection. No parasites detected up to 514th day.

*Result.* No detectable change in infection.

*History of 4th superinfection.* Superinfected on 514th day with same strain. Blood taken from Monkey No. 159 (*S. irus*) (passage 8a) after death.\* No parasites detected until 549th day, after which present in very scanty numbers up to 556th day. (This was possibly due to a relapse of one of the previous infections and not to the last superinfection.)

*Result.* No detectable change in infection. It was, however, doubtful whether the blood injected was infective at the time.

### Results of multiple superinfections with strain C.

*Experiment (iv).*—The primary superinfection caused a very transient appearance of parasites in the peripheral blood, while the next three produced no detectable effects. In two of the latter instances it was doubtful, however, whether the blood was infective at the time of inoculation, although taken from *S. irus*.

This animal is of special interest, in that the 1st superinfection was made after repeated blood examinations, during a period of over one year, had failed to reveal parasites, even when protein shock was used. This may mean either that the animal was cured but still possessed a tolerance to strain C, or that an undetected latent infection was still present.

#### (4) DISCUSSIONS OF THE EFFECTS OF MULTIPLE SUPERINFECTIONS WITH HOMOLOGOUS STRAINS OF *P. KNOWLESI*.

Mulligan and Sinton (1933) found, under the conditions of their experiments, that a chronic or latent infection with one strain of *P. knowlesi* produced a high degree of tolerance to the clinical effects of superinfection with the same strain of parasite. The results recorded in the present investigation support these findings. These have been summarized in the tables given later in this paper.

Although clinical symptoms may not be detectable following many of the homologous superinfections in monkey malaria, yet the effects of these may be manifest in many cases by parasitic relapses of varying intensity. While such relapses would probably be productive of clinical manifestations in human

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\* This animal was splenectomized before being infected with strain C. It developed during the next 3 weeks two very acute attacks, both of which required treatment (quinine and plasmoquine). The animal then developed a very severe anæmia which killed it in about 12 days, in spite of almost daily treatment with stovarsol. No parasites had been detected in the peripheral blood for 12 days before death, but a very few were found on careful search of smears of the spleen and internal organs.

malaria, they are only detectable by very frequent blood examinations in monkeys.

During the work on therapeutic malaria in nervous diseases, several workers have shown that a chronic infection with one strain of *P. vivax* produces a considerable degree of tolerance to superinfection with the same strain (*vide* references in Mulligan and Sinton, 1933). This effect was observed whether the superinfection was caused by blood inoculation or by mosquito bite. The degree of tolerance produced appeared to increase with the number of superinfections given. Slight clinical manifestations may be caused by primary homologous superinfections in human malaria, but get gradually less and are usually absent in the later ones. James, Nicol and Shute (1932) have also recorded a similar increase in tolerance following multiple superinfections with a homologous strain of *P. falciparum*.

It will be noted that in our experiments [Nos. (i) to (iv)] when the same species of donor animal was used (*S. rhesus*), there was a similar tendency for the effects of superinfection to diminish. The results have, therefore, a considerable resemblance to those recorded in human malaria.

This effect may indicate either (a) an increased tolerance of the host to the toxic effects of the same number of parasites, or (b) an increased parasitocidal mechanism of the animal, which enables it to restrain the number of parasites below the detectable 'toxic threshold'.

The investigations recorded by Sinton *et al.* (1931) in chronic benign tertian malaria may indicate that chronically infected patients have developed some tolerance to the 'toxins' of parasite. This was suggested by the apparent occurrence of a higher 'pyrogenous limit' for the prevalence of parasites in such cases, as compared with primary or less chronic infections. The findings reported by the above authors might, however, be explicable on the assumption that there was a decrease in the virulence of the parasite, as the result of the prolonged action of the defensive mechanism of the host.

While these factors probably play some rôle in acquired tolerance, it seems more likely that the parasitocidal mechanism is more important. The numerical prevalence of the parasites in the peripheral blood, during the relapses of primary infections, is usually observed to become successively less. A similar effect appears to take place in the case of successive superinfections with homologous strains. This shows that there must be a powerful stimulation to the parasitocidal mechanism.

#### (a) Possible changes in the virulence of a strain of parasite.\*

Boyd (1925) found evidence to suggest that in bird malaria the parasites taken during the first few days of an acute infection were more virulent than

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\* 'Virulence' is merely a comparative term, and seems to have been applied to two different conditions. (a) It may mean that varying degrees of toxic effect, as judged by the clinical manifestations, may be caused by parasitic infections of the same intensity.

those derived from a chronic one. He also thought the virulence of the parasites appeared to decrease to a slight extent as an infection progressed. This author evaluated the degree of virulence in his infections by 'variations in the readiness with which the parasites are able to invade a new host, the severity of the infections produced, and the death rate they are capable of bringing about'.

All the strains of *P. knowlesi* used in our work have caused such severe and fatal infections that we have usually found it impossible to detect any slight differences in the virulence of the primary infections. In the case of strain K<sub>4</sub>, however, the relapses following the primary infection appear to be more severe, more frequent, and to require therapeutic control more often, than in any of the other strains. This strain has also proved more difficult to establish as a chronic infection, because of the numerous deaths during relapses. These observations appear to indicate a higher degree of virulence in this strain, or a diminished power of *S. rhesus* to produce tolerance against it.

In contradistinction to the findings of Boyd (1925), we have been unable to detect any differences in the severity of infections produced by blood obtained from chronic infections as compared with that from acute ones. This result refers both to primary infections and to superinfections with homologous strains.

Boyd (1925) also thought that the diminished virulence of his strain in chronic infections could be increased again, somewhat quickly, by rapid passage from bird to bird. When the virulence attained a certain degree, however, no further enhancement was observed. Napier and Campbell (1932) and Knowles and Das Gupta (1932) also believed that repeated passages of *P. knowlesi* (strain C) through *S. rhesus* caused an increase in virulence. This was shown by a shorter period of incubation and the more frequent occurrence of hæmoglobinuria. One might expect such a change in virulence to occur, when a parasite is passaged from a relatively tolerant host (*S. irus*) into a much more susceptible one (*S. rhesus*). On the other hand, it might be argued that in the more tolerant host it would only be the more virulent strains which would survive.

On account of the great susceptibility of *S. rhesus* to primary infections with *P. knowlesi*, it was not found possible to be certain of slight changes in

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As was pointed out by Sinton *et al.* (1931) the reaction of the host to the same number of parasites depends not only upon the individual susceptibility of the animal, but also upon the presence of any tolerance, either natural or acquired, to the strain of parasite causing the infection. (b) On the other hand, in some instances, the degree of virulence of a parasite has been judged by its ability to multiply in the host. This may not be proportionate to the toxic effects produced. The degree of virulence in the latter case is judged not by the effects produced on the host, but on the ability of the parasite to multiply under the conditions present in the host. Slight variations in the toxic effects are more difficult to evaluate in the animal host as compared with the human one. It has, therefore, been necessary in the case of superinfections in monkeys to place more reliance upon differences in the degree of parasite prevalence.

virulence as the result of passage. It was found to be easier to judge of such variations in animals which have been allowed to develop some tolerance as the results of a previous infection. The results obtained in experiments (i), (ii) and (iii) suggest that strains derived from *S. rhesus* are less virulent than those from *S. irus*, as judged by the effects produced by homologous superinfections. Strains inoculated from *S. irus* appeared to produce more marked parasitic relapses during such superinfections than did those from *S. rhesus*. These findings were not, however, confirmed by the results obtained in experiment (iv), but in the latter experiment some doubt exists as to the infectivity of the blood inoculated from *S. irus*. The matter requires further investigation.

These findings suggest the following explanations, either that

(a) the fewer passages a strain is distant from the original host by blood inoculation, the more virulent it is, or that

(b) the strains in *S. irus* have a higher degree of virulence than those in *S. rhesus*, but that subpassage again to the former species tends to raise any lowered virulence, or that

(c) some of our strains were not pure but were composed of two or more strains of different virulence, some of which had been eliminated during frequent passage in *S. rhesus*.

The results at present available are too few on which to draw any definite conclusions upon the questions raised. It is hoped that later experiments will shed more light on these problems.

#### (b) The occurrence of composite strains.

The results obtained suggest, in several instances, that some of the strains used originally were mixed ones, and that some of the integral components of these had been eliminated during the course of repeated passages or prolonged chronic infection. Such portions might be eliminated by

(a) not being present in a viable state at the time the blood was taken for inoculation, or

(b) by the therapeutic measures employed in some cases, or

(c) by the natural defences of the body in others, or

(d) by a combination of these factors.

The elimination of any one component strain would result in a gradual loss of tolerance to that strain, so that reinoculation with the original mixed strain would produce a more marked parasitic increase than might a superinfection with the same depleted strain from a chronic infection.

There is some evidence to suggest that some of the strains of *P. knowlesi* used in our investigations might be of such a composite nature (*vide infra*, Discussion on heterologous superinfections).

Multiple superinfections with a homologous strain appear to produce a higher degree of tolerance than a single infection lasting over the same period of time. This also suggests that some element in the primary infection has either died out, or has lost some of its power of producing a continued tolerance.

**(c) Differences in the immunological properties of parasites taken from acute as compared with chronic infections.**

Several workers on relapsing fever have reported that the strain of parasite isolated during the relapses has different serological properties from that found in the primary attack. It appeared possible that a similar phenomenon might be present in a relapsing infection like malaria.

The second and third homologous superinfections recorded in experiment (iv) were made with blood taken during relapses of chronic infections. These relapses followed upon splenectomy. In neither of these superinfections was any apparent change produced in the infection. The results obtained in experiments (i) and (ii) also give no indication that the strains were different when taken from an acute primary infection, or from a chronic one after several relapses had occurred.

In experiment (iii) the primary superinfection was made from a chronic infection in an animal which had previously been superinfected twice with a homologous strain. No difference in virulence was detected in the result under these conditions.

We have, therefore, been unable to find any evidence to support the suggestion that the immunological properties of a strain, in the same species of host, are very markedly different in the primary infection, as compared with that in relapses. No change in the strain appears to be produced as the result of several homologous superinfections.

**(5) SUMMARY OF THE RESULTS OF MULTIPLE HOMOLOGOUS SUPERINFECTIONS.**

Under the conditions of our experiments it was found that—

(a) A simple chronic or latent infection with one strain of *P. knowlesi* produced a considerable degree of tolerance to the clinical effects of superinfection with the same strain.

(b) The results of homologous superinfections are generally of such a mild nature that clinical manifestations, apart from the occasional occurrence of anæmia, are usually absent in monkeys. The effects of homologous superinfection, when detectable, are shown by increases in the prevalence of parasites in the peripheral blood.

(c) The effects of homologous superinfection appear to become less evident with each successive superinfection from the same species of host (*S. rhesus*). On the other hand, homologous superinfections made from *S. irus*, during the course of a series of superinfections from *S. rhesus*, usually appear to have more marked effects.

(d) No evidence has been found to suggest that a strain derived from an acute infection is more virulent than, or has different immunological characters from, the same strain obtained from a chronic or a relapsing infection.

(e) There is some evidence to suggest that, in some instances, the strains of *P. knowlesi* used in our work were composed of several different elements of varying degrees of virulence, or of varying powers of stimulating tolerance.

(f) Multiple superinfections with a homologous strain do not appear to produce an effective protection against the clinical effects of a subsequent heterologous superinfection in all cases.

## SECTION II.

### EFFECTS OF MULTIPLE SUPERINFECTIONS WITH HETEROLOGOUS STRAINS.

Five specimens of *S. rhesus* and one of *S. sinicus* have each been superinfected from three to five times with various different strains of *P. knowlesi*. The results of these experiments are summarized in Tables I and II, and the protocols are given below.

#### (v) Monkey No. 7 (*S. rhesus*).\*

*History of primary infection.* Infected by blood inoculation from Monkey No. 2 (*S. rhesus*) (passage 1a) suffering from an acute infection with strain C. Parasites detected on 3rd day; severe attack developed, rapidly reaching maximum on 7th day, when animal showed pernicious symptoms; parasites very numerous on 6th to 11th days; quinine treatment given on 6th, 7th and 8th days; acute relapse occurred on 20th day, but recovery was spontaneous; subsequently infection became chronic with scanty parasites on and off until 341st day, during which period the monkey appeared to be in excellent health.

*Result.* A severe attack requiring treatment; followed by a relapse.

*History of 1st superinfection.* Superinfected on 343rd day with same strain (C). Blood taken from Monkey No. 100 (*S. rhesus*) (passage 4a) during the acute primary attack. Scanty parasites observed on 350th, 351st, 353rd and 354th days; blood examined on 11 occasions up to 379th day, but parasites found on only one occasion (371st day); no clinical symptoms noted after superinfection.

*Result.* No change detected in existing infection.

*History of 2nd superinfection.* Superinfected on 379th day with strain K<sub>1</sub>. Blood taken from Monkey No. 99 (*S. rhesus*) (passage 6) during a severe recrudescence of primary infection. Parasites detected on 384th day; acute attack of great severity developed, becoming maximal on 385th day; recovery followed treatment (plasmoquine and quinine) on 388th, 389th and 390th days; no relapses occurred within an observation period (daily blood examinations) extending to 426th day, but parasites seen in scanty numbers on many occasions.

*Result.* A very severe attack requiring therapeutic control. No relapse occurred.

*History of 3rd superinfection.* Superinfected on 427th day with strain K<sub>1</sub>. Blood taken from old chronic infection after homologous superinfection of Monkey No. 64 (*S. rhesus*) (passages 1 and 3) [vide experiment (ii)]. Parasites markedly increased from 427th to 436th days, then very scanty at daily examinations up to 457th day; afterwards absent till 472nd day, when slight parasitic relapse occurred up to 482nd day; later very scanty parasites occasionally seen up to 494th day.

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\* This animal is the one used in experiments (a) and (j) by Mulligan and Sinton (1933).



TABLE II.  
Summary of results of multiple heterologous superinfections with *P. knowlesi*.

Experiment number.	FIRST HETEROLOGOUS SUPERINFECTION.			SECOND HETEROLOGOUS SUPERINFECTION.			THIRD HETEROLOGOUS SUPERINFECTION.			FOURTH HETEROLOGOUS SUPERINFECTION.			FIFTH HETEROLOGOUS SUPERINFECTION.		
	Primary infection.	Superinfection.	Result.	Previous infections.	Fresh superinfection.	Result.	Previous infections.	Fresh superinfection.	Result.	Previous infections.	Fresh superinfection.	Result.	Previous infections.	Fresh superinfection.	Result.
(i)	2C*	K <sub>1</sub>	++	2C+K <sub>1</sub>	K <sub>2</sub>	+	2C+K <sub>1</sub> +K <sub>2</sub>	K <sub>3</sub>	-	2C+K <sub>1</sub> +K <sub>2</sub> +K <sub>3</sub>	K <sub>4</sub>	+			
(ii)	2C*	K <sub>1</sub>	++	2C+K <sub>1</sub>	K <sub>2</sub>	±	2C+K <sub>1</sub> +K <sub>2</sub>	K <sub>3</sub>	+	2C+K <sub>1</sub> +K <sub>2</sub> +K <sub>3</sub>	K <sub>4</sub>	+			
(iii)	C	K <sub>1</sub>	++	C+K <sub>1</sub>	K <sub>2</sub>	+	C+K <sub>1</sub> +K <sub>2</sub>	K <sub>3</sub>	±	C+K <sub>1</sub> +K <sub>2</sub> +K <sub>3</sub>	K <sub>4</sub>	+	C+K <sub>1</sub> +K <sub>2</sub> +K <sub>3</sub> +K <sub>4</sub>	K <sub>5</sub>	+++
(iv)	C	K <sub>2</sub>	+++	C+K <sub>2</sub>	K <sub>1</sub>	+	C+K <sub>2</sub> +K <sub>1</sub>	K <sub>3</sub>	+	C+K <sub>2</sub> +K <sub>1</sub> +K <sub>3</sub>	K <sub>4</sub>	±			
(v)	K <sub>2</sub>	K <sub>1</sub>	++	K <sub>2</sub> +K <sub>1</sub>	C	-	K <sub>2</sub> +K <sub>1</sub> +C	K <sub>3</sub>	±						
(z)	K <sub>2</sub>	K <sub>1</sub>	++	K <sub>2</sub> +K <sub>1</sub>	K <sub>3</sub>	±	K <sub>2</sub> +K <sub>1</sub> +K <sub>3</sub>	K <sub>4</sub>	+						

\* These animals had previously received a homologous superinfection.

**Result.** A distinct, but not severe, parasitic infection resulted, followed by a slight parasitic relapse later.

**History of 4th superinfection.** Superinfected on 494th day with strain K<sub>1</sub>. Blood taken during primary acute attack in Monkey No. 171 (*S. rhesus*)\* (passage 1). Very scanty parasites seen on most days from 495th till 512th days, then absent, except twice, till 523rd day.

**Result.** Practically no change in infection.

**History of 5th superinfection.** Superinfected on 523rd day with strain K<sub>4</sub>. Blood taken from old chronic superinfection in Monkey No. 110 (*S. rhesus*) (passages 2 and 3) [vide experiment (f), Mulligan and Sinton, 1933]. Parasites detected from 524th day onwards, with marked increase from 525th to 530th days from 534th to 542nd days and from 548th to 550th days, diminishing in the intervals. A distinct parasitic relapse due to *P. knowlesi* occurred from 563rd to 573rd days, after which parasites again became scanty. A marked parasitic relapse due to *P. cynomolgi* occurred between 580th and 588th days. (The latter was probably the result of the 4th superinfection). This was quickly followed by another slight parasitic relapse of *P. knowlesi*.

**Result.** A definite parasitic attack without clinical symptoms, followed by several distinct parasitic relapses.

### Summary of results of experiment (v).

A superinfection with strain C superimposed on a prolonged chronic infection with the same strain proved insufficient to prevent a very severe attack requiring treatment, when the animal was again superinfected with a heterologous strain (K<sub>1</sub>). A later superinfection with strain K<sub>2</sub> produced a distinct parasitic relapse, while a 4th superinfection with strain K<sub>3</sub> was without effect. In spite of the duration of the experiment and the number of heterologous superinfections, the tolerance produced was not sufficient to prevent a distinct parasitic relapse after a subsequent superinfection with strain K<sub>4</sub>. The latter strain showed its usual tendency to relapse.

These results suggest that strains C and K<sub>1</sub> are heterologous, but that superinfection with these two strains produces a considerable degree of tolerance against strains K<sub>2</sub> and K<sub>3</sub>. Strain K<sub>4</sub> probably contains some element heterologous to the other four strains used. This would not be surprising as it was isolated from a different batch of animals, purchased at a different time.

### (vi) Monkey No. 29 (*S. rhesus*).†

**History of primary infection.** Infected by blood inoculation with strain C from Monkey No. 28 (*S. rhesus*) (passage 9a). Blood taken at time of acute infection. Parasites detected on 4th day and increased rapidly to reach maximum on 6th day; severe attack with pernicious symptoms; quinine treatment given on 6th and 7th days arrested attack; severe relapse occurred on 11th day, but recovery followed quinine given on 12th day; no further relapses of a severe nature occurred, and animal acquired a chronic infection with periodical appearance of scanty parasites

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\*This animal had a mixed infection of *P. knowlesi* and *P. cynomolgi*.

†This monkey is the one used in experiments (b) and (k) by Mulligan and Sinton (1933).

until 152nd day; subsequently no parasites were observed (irregular examinations) up to 257th day.

**Result.** A very severe attack, and also a relapse, which required treatment.

**History of 1st superinfection.** Superinfected on 258th day with same strain (C). Blood taken from old chronic infection in Monkey No. 21 (*S. rhesus*) (passage 5a). Parasites reappeared on 260th day and were observed daily in scanty numbers until 269th day; parasites were relatively more abundant on 265th day; examinations made almost daily up to 301st day revealed parasites on only two subsequent occasions.

**Result.** No acute attack, but a very slight transient increase in the number of parasites.

**History of 2nd superinfection.** Superinfected on 309th day with strain K. Blood taken from Monkey No. 99 (*S. rhesus*) (passage 6) at the end of acute relapse. Parasites detected on 314th day and very acute attack developed quickly, becoming maximal on 317th day; plasmoquine treatment was given on 317th and 318th days, and the animal recovered; no clinical relapse occurred during an observation period extending to 356th day (daily blood examinations), but scanty parasites found on all but 2 days.

**Result.** A very severe attack with very high parasite counts and serious clinical symptoms requiring treatment, followed by a prolonged parasitic infection.

**History of 3rd superinfection.** Superinfected on 357th day with strain K. Blood taken during chronic superinfection in Monkey No. 64 (*S. rhesus*) (passages 1 and 3) [vide experiment (c), Mulligan and Sinton, 1933]. Few parasites detected on 360th day, increasing to moderate numbers on 366th and 367th days; later very scanty or absent up to 383rd day. Negative at daily examinations up to 423rd day, except for few on 408th and 412th days.

**Result.** Very slight increase in parasites.

**History of 4th superinfection.** Superinfected on 423rd day with strain K. Blood taken from Monkey No. 171\* (*S. rhesus*) (passage 1) during acute primary attack. Parasites detected on 427th day and remained scanty till 434th day, when a moderately severe parasitic relapse of *P. knowlesi* occurred lasting till 439th day; later parasites gradually became scanty or absent till 452nd day.

**Result.** A parasitic attack of moderate severity occurred.

**History of 5th superinfection.** Superinfected on 452nd day with strain K. Blood taken from Monkey No. 110 (*S. rhesus*) (passages 2 and 3), during course of chronic homologous superinfection. Parasites increased in number from 454th to 462nd days, then decreased again and were absent from 465th to 476th days. A distinct parasitic relapse due to *P. cynomolgi* then occurred, reaching a maximum about 484th day and gradually diminishing. A mixed infection with *P. knowlesi* and *P. cynomolgi* was detectable at most daily examinations from 490th to 496th days, but the former species quickly predominated and caused a distinct parasitic relapse from this time up to 504th day. Parasites then rapidly diminished and were absent from 509th to 514th days, after which a marked parasitic relapse of *P. cynomolgi* occurred and this parasite was present up to 527th day.

**Result.** Superinfection was followed by a distinct attack due to *P. knowlesi*; later relapses due both to this *Plasmodium* and to *P. cynomolgi* occurred.

### Summary of results of experiment (vi).

The infection and superinfections in this experiment were identical with those of the previous one. The intervals between the superinfections were,

\* This monkey was suffering from a mixed infection of *P. knowlesi* and *P. cynomolgi*.

however, shorter in some cases. The results obtained were very similar, except that strain K<sub>3</sub> caused slightly more marked effects and strain K<sub>2</sub> slightly less. In this experiment also, superinfection with strain K<sub>4</sub> caused a distinct rise in the number of parasites at the 5th superinfection. The tendency of this strain to relapse was well marked.

**(vii) Monkey No. 9 (*S. rhesus*).\***

*History of primary infection.* Infected with strain C by blood inoculation from Monkey No. 2 (*S. rhesus*) (passage 1a). Blood taken during the course of an acute infection. Parasites detected on 5th day and increased rapidly; acute attack of extreme severity, becoming maximal on 9th day when animal showed severe pernicious symptoms, including hæmoglobinuria; quinine given on 8th, 9th and 10th days, after which recovery occurred; severe relapse on 19th day, stopped by a single dose of quinine; subsequently two relapses occurred on 131st and 180th days, but both recovered spontaneously; infection then became chronic with periodical appearance of parasites until 310th day; later no parasites observed (irregular examinations) up to time of superinfection.

*Result.* An extremely severe acute attack requiring treatment; several clinical relapses.

*History of 1st superinfection.* Superinfected on 343rd day with strain K<sub>1</sub>. Blood taken from chronic infection of Monkey No. 74 (*S. rhesus*) (passage 5). Parasites detected on 347th day and increased rapidly; acute attack of moderate severity becoming maximal on 350th day; parasites decreased in numbers on 352nd and 353rd days but increased again on 354th, subsequently decreasing to scanty numbers without treatment; few or very few parasites present at daily examination up to 378th day, no relapse observed.

*Result.* A moderately acute attack with spontaneous recovery; no relapse.

*History of 2nd superinfection.* Superinfected on 379th day with strain K<sub>1</sub>. Blood taken during acute relapse in Monkey No. 98 (*S. rhesus*) (passage 2). Scanty parasites seen on 380th and 381st days, then distinct increase during period 382nd to 396th days; parasites later found in small numbers up to 403rd day, when animal became very anæmic and given stovarsol (1 grain) on last day. Daily blood examinations showed very scanty or no parasites up to 427th day.

*Result.* A distinct parasitic attack followed by anæmia requiring treatment; no later relapses during observation.

*History of 3rd superinfection.* Superinfected on 427th day with strain K<sub>2</sub>. Blood taken from Monkey No. 56 (*S. irus*) (original host). Parasites very few or scanty at daily examinations up to 484th day, except for distinct increases at 448th to 450th days, at 456th and 457th days, and definite parasitic relapse at 473rd to 481st days.

*Result.* Slight parasitic relapses.

*History of 4th superinfection.* Superinfected on 484th day with strain K<sub>4</sub>. Blood taken during chronic superinfection in Monkey No. 110 (*S. rhesus*) (passages 2 and 3) [vide experiment (f) of Mulligan and Sinton, 1933]. Parasites detected on 490th day and remained scanty till 494th day; after which considerable increase lasting till 509th day, with marked exacerbation at 506th day; later daily examinations only twice positive up to 523rd day.

*Result.* Parasitic attack of moderate severity.

\* This is the animal used in experiment (h) by Mulligan and Sinton (1933).

**History of 5th superinfection.** Superinfected on 523rd day with strain K<sub>5</sub>. Blood taken from Monkey No. 162 (*S. irus*) (original host). Few parasites detected next day, these increased very rapidly and, in spite of treatment (plasmoquine and quinine) on 529th day, the animal died.

**Result.** A fatal result, in spite of tolerance produced by previous superinfections with four different strains of *P. knowlesi* and a chronic infection lasting over 523 days.

### Summary of results of experiment (vii).

The primary infection was one of strain C and a superinfection with strain K<sub>1</sub> caused an acute clinical relapse, which recovered spontaneously. A second superinfection with strain K<sub>2</sub> caused a distinct parasitic relapse with some anaemia, while a later superinfection with strain K<sub>3</sub> produced a very slight effect. As in the previous experiments strain K<sub>4</sub> caused a distinct parasitic relapse. It might have been expected that a very high degree of tolerance would have been produced in an animal which had been infected and superinfected with five strains of *P. knowlesi*, and in which a chronic infection had lasted for 17 months. A fifth superinfection was made with strain K<sub>5</sub> on 523rd day and this proved rapidly fatal in spite of treatment. Unfortunately we have no other result with which to compare the effects of superinfection with the latter strain, except that in *S. sinicus* used in experiment (x). The latter species of monkey appears to be much less susceptible to *P. knowlesi* than is *S. rhesus*, so the results are not quite comparable.

The interval between the last two superinfections in this case was about 40 days. It is impossible to be certain that the severe effects of strain K<sub>5</sub> were not attributable, partly at least, to a concurrent relapse of strain K<sub>4</sub> superimposed on an acute infection with the former strain. Strain K<sub>4</sub> has been found to be more virulent than any of the other strains investigated so far, and it has also shown a greater tendency to relapse.

### (viii) Monkey No. 39 (*S. rhesus*).\*

**History of primary infection.** Infected with C strain by blood inoculation from Monkey No. 40 (*S. rhesus*) (passage 2a). Blood taken during course of acute infection under treatment. Parasites detected on 8th day, and increased rapidly to reach maximum on 11th day; resultant attack very severe with pernicious symptoms; recovery followed tebetren treatment given daily from 9th to 19th days; no severe relapse observed; a low-grade infection developed during which parasites in scanty numbers seen from time to time up to 195th day.

**Result.** A very severe attack controlled by treatment.

**History of 1st superinfection.** Superinfected on 203rd day with strain K<sub>1</sub>. Blood taken from a chronic superinfection in Monkey No. 64 (*S. rhesus*) (passages 1 and 3) [vide experiment (e) of Mulligan and Sinton, 1933]. Parasites detected on 216th day and became maximal on 222nd day; acute attack with marked symptoms developed and was controlled by quinine and stovarsol given on 222nd, 223rd and 224th days; no subsequent relapses observed, but parasites usually present in scanty numbers (23 examinations) up to 286th day.

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\*This animal is that used in experiment (I) by Mulligan and Sinton (1933).

**Result.** An acute attack of considerable severity controlled by treatment.

**History of 2nd superinfection.** Superinfected on 287th day with strain K<sub>1</sub>. Blood taken from chronic infection in Monkey No. 55 (*S. irus*) (passage 2). Parasites detected on 296th day, rising to moderately severe parasitic infection on 297th to 299th days; animal became very anæmic and stovarsol (1 grain) given on 300th day. Parasites mostly few till 323rd day and thereafter not detected till 333rd day; then again increased till 337th day and later none found up to 343rd day.

**Result.** Moderately severe parasitic attack with relapse of a slight nature; some anæmia after attack.

**History of 3rd superinfection.** Superinfected on 343rd day with strain K<sub>1</sub>. Blood taken from chronic superinfection in Monkey No. 110 (*S. rhesus*) (passages 2 and 3) [vide experiment (f) of Mulligan and Sinton, 1933]. Few parasites detected from 351st to 354th days; distinct rise in numbers from 355th to 366th days. Later daily examinations showed scanty parasites on two occasions up to 384th day.

**Result.** A distinct parasitic relapse.

**History of 4th superinfection.** Superinfected on 384th day with strain K<sub>1</sub>. Blood taken from Monkey No. 128 (*S. irus*) (original host).<sup>\*</sup> Slight transient rise in number of parasites, with maximum about 393rd day; parasites absent or very few from 396th to 400th days, and not seen again up to 422nd day. A slight parasitic relapse due to *P. knowlesi* then occurred with its maximum on 427th day; parasites absent from 431st to 457th day.

**Result.** A very slight parasitic relapse due to *P. knowlesi*.

### Summary of results of experiment (viii).

Superinfection with strain K<sub>2</sub> upon a chronic infection with strain C caused a very severe attack. Subsequent superinfections with strains K<sub>1</sub> and K<sub>4</sub> produced definite parasitic relapses without any clinical symptoms. A subsequent superinfection with strain K<sub>5</sub> was only followed by a slight increase in the parasite prevalence.

### (ix) Monkey No. 80 (*S. rhesus*).†

**History of primary infection.** Infected with strain K<sub>2</sub> by blood inoculation from chronic infection in Monkey No. 56 (*S. irus*) (original host). Parasites detected on 8th day; quinine given on 9th and 10th days controlled progress of attack temporarily; subsequent severe attack developed (14th to 18th days), but recovery followed treatment (quinine and plasmoquine) given on 14th, 17th, 19th and 22nd days; relapse observed on 31st day, but recovery spontaneous; no further relapse occurred, but parasites seen periodically in scanty numbers till 125th day.

**Result.** Severe attack requiring treatment, followed by a relapse.

**History of 1st superinfection.** Superinfected on 126th day with strain Cyn<sub>1</sub> (*P. cynomolgi*). Blood taken from Monkey No. 97 (*S. rhesus*) (passage 2) during primary acute attack. Heavy parasitic infection with *P. cynomolgi* developed; recovery spontaneous with subsequent low-grade infection with both species of parasite up to 206th day.

<sup>\*</sup> This monkey was suffering from a mixed infection of *P. knowlesi* and *P. cynomolgi*.

† This animal is the one used in experiments (s) and (v) of Mulligan and Sinton (1933).

**Result.** Severe attack due to *P. cynomolgi*, followed by spontaneous recovery.

**History of 2nd superinfection.** Superinfected on 207th day with strain K<sub>1</sub>. Blood taken from chronic infection in Monkey No. 55 (*S. irus*) (passage 2). *P. cynomolgi* present at time of superinfection; acute attack due to *P. knowlesi* developed on 224th day; quinine treatment required for 1 day; scanty parasites of latter species present up to 253rd day with parasitic relapse about 250th day; later mixed infection of varying but slight intensity present up to 274th day.

**Result.** A moderately severe attack requiring treatment; very slight parasitic relapse due to *P. knowlesi*.

**History of 3rd superinfection.** Superinfected on 274th day with strain C. Blood taken during acute primary attack in Monkey No. 167 (*S. rhesus*) (Passage 6a). Daily blood examinations negative, except for very few parasites on five occasions up to 296th day; few parasites seen from 297th to 304th days.

**Result.** No very appreciable change in infection.

**History of 4th superinfection.** Superinfected on 305th day with strain K<sub>1</sub>. Blood taken from Monkey No. 132 (*S. rhesus*) (passage 2) during a chronic infection. Slight transient increase of parasites (*P. knowlesi*) from 311th to 315th days; very few or absent from 317th to 329th days; then marked relapse due to *P. cynomolgi* from 330th to 336th days, with maximum about 333rd day. A slight parasitic relapse due to *P. knowlesi* again occurred from 348th to 350th days and a more marked one from 365th to 380th days; parasites absent in intervals.

**Result.** Transient parasitic relapses due to *P. knowlesi* and also one of *P. cynomolgi*.

### Summary of results of experiment (ix).

A superinfection of *P. cynomolgi* (strain Cyn<sub>1</sub>) on a chronic infection with strain K<sub>2</sub> of *P. knowlesi* caused an acute clinical attack, which recovered spontaneously. A later superinfection with *P. knowlesi* (strain K<sub>1</sub>) and *P. cynomolgi* from a mixed infection in *S. irus* was followed by severe attack due to the former parasite. This attack required treatment. A third superinfection with strain C had no appreciable effect. The fourth superinfection with strain K<sub>2</sub> produced but a transient increase in the number of *P. knowlesi*, a marked parasitic relapse of *P. cynomolgi* occurred, however, after the later relapse. This relapse may have been due either to the infection introduced at the 1st superinfection (200 days before), or from the mixed infection at the 2nd superinfection (about 100 days previously), probably the latter.

The results with *P. knowlesi* suggest that strains K<sub>1</sub> and K<sub>2</sub> may contain some of the immunological elements present in strain C.

### (x) Monkey No. 82 (*S. sinicus*)\*

**History of primary infection.** Infected with strain K<sub>1</sub> by blood inoculation from Monkey No. 77 (*S. rhesus*) (passage 2). Blood taken during course of acute infection. Parasites detected on 5th day; very numerous plasmodia associated with severe constitutional symptoms observed on 8th day; treatment (quinine and plasmoquine) given on 8th and 9th days, arrested acute attack and blood became free from parasites on 17th and 18th days; no relapse occurred and

\* This is the animal used in experiment (r) by Mulligan and Sinton (1933).

monkey remained healthy, although parasites found in scanty numbers until 119th day; none found thereafter (six examinations) up to 172nd day.

*Result.* A severe primary attack requiring treatment, but no relapse.

*History of 1st superinfection.* Superinfected on 173rd day with strain K<sub>1</sub>. Blood taken from chronic infection in Monkey No. 99 (*S. rhesus*) (passage 6). Parasites detected on 180th day and moderately heavy infection ensued, reaching maximum on 189th day; no treatment given; acute attack subsided spontaneously; parasites disappeared on 191st day; stovarsol given on 189th day, as monkey very anæmic; parasites seen occasionally in scanty numbers up to 224th day, then negative at weekly examinations up to 246th day.

*Result.* An acute infection followed by spontaneous recovery.

*History of 2nd superinfection.* Superinfected on 246th day with strain K<sub>4</sub>. Blood taken from chronic superinfection in Monkey No. 110 (*S. rhesus*) (passages 2 and 3) [vide experiment (f) of Mulligan and Sinton, 1933]. Very few parasites detected on 252nd and 253rd days, but distinct increase during next two days; later negative at daily examinations up to 296th day except for very few parasites on six occasions.

*Result.* A very transient parasitic rise.

*History of 3rd superinfection.* Superinfected on 296th day with strain K<sub>6</sub>. Blood taken from Monkey No. 162 (*S. irus*) (original host). Scanty parasites detected from 305th to 307th days; marked increase with maximum on 310th to 312th days; later gradual decrease, parasites remaining very few or absent till 320th day; slight rise from 320th to 323rd days, and absent from 324th till 364th day.

*Result.* A definite parasitic relapse without clinical symptoms.

### Summary of results of experiment (x).

As this animal was a specimen of *S. sinicus*, a more tolerant host, the results obtained are not strictly comparable with those recorded with *S. rhesus*. A chronic infection with strain K<sub>2</sub> was insufficient to prevent an acute clinical attack following a superinfection with strain K<sub>1</sub>. This infection was, however, modified and recovered spontaneously. A second superinfection with strain K<sub>4</sub> produced but a slight increase in the number of parasites, while a third with strain K<sub>6</sub> caused a distinct increase. This animal should be compared with *S. rhesus* in experiment (vii). The latter received the same superinfections and in addition strains C and K<sub>3</sub> during a longer period. The specimen of *S. rhesus*, however, died in a very acute attack following the last superinfection (strain K<sub>6</sub>). This adds additional support to the conclusion reached by Mulligan and Sinton (1933) that *S. sinicus* is less susceptible to *P. knowlesi* than is *S. rhesus*.

### DISCUSSION OF THE EFFECTS OF MULTIPLE SUPERINFECTIONS WITH HETEROLOGOUS STRAINS OF *P. KNOWLESI*.

Mulligan and Sinton (1933) found, under the conditions of their experiments, that a chronic or latent infection with one strain of *P. knowlesi* appeared to confer some tolerance to the clinical effects of superinfection with a different strain of the same parasite. This was indicated by an increased tendency for the acute initial attack of the superinfection to recover spontaneously, and by



a diminished tendency for such infections to relapse clinically at a later date. This tolerance appeared to be mainly specific, but there was some evidence to suggest that some common immunizing power existed between the strains of the parasite used by them. This was possibly due to a general stimulation of the reticulo-endothelial system by the protozoal infection.

In man and birds, several workers have recorded the effects of superinfection with a single heterologous strain of *Plasmodium* (*vide* summary given by Mulligan and Sinton, 1933). Gingrich (1932) seems to be the only worker who has studied the effects of more than one heterologous strain of the same parasite. This worker reports that a latent or chronic infection of any of the five strains of *P. relictum* used by him, is associated with an effective immunity to superinfection with any other strain of this parasite. This tolerance to heterologous superinfection in birds is markedly different from the absence of tolerance reported in chronic or latent plasmodial infections in man and monkeys.

It was noted in our earlier work that the effects of a heterologous superinfection were apparently milder than those of the primary infection. It was, therefore, thought advisable to investigate the effects of further heterologous superinfections on animals which had previously had only a single one. It seemed possible that multiple superimposed heterologous superinfections might eventually produce a very effective degree of tolerance to all other strains of the same parasite. The results of these experiments have been summarized in Tables I and II.

**(a) The results of multiple homologous superinfections on the effects of subsequent heterologous superinfections.**

The results recorded in experiments (v) and (vi) do not indicate that a single homologous superinfection produces any greater tolerance to a subsequent heterologous superinfection than was produced by a simple chronic infection.

In experiments (i) and (ii) four homologous superinfections were given during the course of one year. These superinfections included inoculations from both acute and chronic infections in *S. rhesus*, and from chronic infections in *S. trus*. At the end of one year these animals were superinfected with the same heterologous strain at the same time. In experiment (i) a severe clinical attack with spontaneous recovery occurred when strain C was superinfected on strain K<sub>1</sub>. The incubation period was, however, prolonged. On the other hand, when the same strain was superinfected on strain K<sub>2</sub>, a very severe and fatal infection resulted. These results indicate that multiple superinfections with a homologous strain of *P. knowlesi* will not protect in all cases against a subsequent heterologous superinfection with the same parasite.

The prolonged incubation period recorded in experiment (i) suggests some similarity between strains C and K<sub>1</sub> in respect of immunological properties. They also suggest that the relationship between these two strains is closer than between strains C and K<sub>2</sub>. Such a view is supported to some extent by the

results obtained by Mulligan and Sinton (1933) in simple heterologous superinfections with these strains.

Further work along these lines is being carried out.

**(b) The results of multiple heterologous superinfections on the effects of subsequent heterologous superinfections.**

The results of these superinfections are summarized in experiments (v) to (x). These, taken in conjunction with those of Mulligan and Sinton (1933), show that an infection with strain C does not confer any very effective tolerance against the clinical and parasitic effects of superinfection with either strains  $K_1$  or  $K_2$ . This indicates that the two latter strains have some immunological elements different from strain C. Such a result was not unexpected as strain C was isolated from a different consignment of animals, and at a different time from the other two strains.

The results also show that infections with either strain  $K_1$ ,  $K_2$  or  $K_3$  do not protect against heterologous infections with either strain  $K_1$  or  $K_2$ . The results of the latter superinfections would in some instances appear to be slightly milder than those of heterologous superinfections with strain C and *vice versa*. This suggests that the first three strains may have some immunological factor in common. Such a suggestion is not improbable as they were all isolated from the same batch of monkeys, which possibly came from the same locality and were thus subject to the same risks of infection in nature. The fact that mixed infections are apparently common in *S. irus* shows that these animals are liable to contract multiple infections in nature (Sinton and Mulligan, 1933). Under such circumstances, it would not be surprising if some animals became infected with mixed infections of the same strains in varying combinations.

Such a possibility is also suggested by the observation that infection and superinfection with strains C and  $K_1$  respectively produce a considerable degree of tolerance to a later superinfection with strain  $K_2$  [experiments (v), (vi) and (vii)]. Infection and superinfection with strains C and  $K_2$  respectively also caused some tolerance against strain  $K_1$  [experiment (viii)]. A superinfection of strain C, on strains  $K_3$  and  $K_1$  combined, produced no effect [experiment (ix)]. The latter observation suggests that these combined strains include the main immunological factors present in strain C. That strain  $K_1$  may contain some of the immunological factors of strain C appears to be indicated by experiment (i).

Superinfection of strain  $K_3$ , on strains C,  $K_1$  and  $K_2$  combined, produced little effect [experiments (v), (vi) and (vii)]. All these findings suggest that strains  $K_1$ ,  $K_2$  and  $K_3$  contain some common immunological factors, but that the combination of such factors is different in each strain. Similarly it seems probable that these three strains together contain most of the immunological factors present in strain C. The permutations and combinations of the factors

in these four strains are probably numerous, and would require many complicated experiments to determine.

The monkeys in experiments (v), (vi) and (vii) had all suffered from a primary infection with strain C, and with heterologous superinfections with strains K<sub>1</sub>, K<sub>2</sub> and K<sub>3</sub> during periods from 344 to 523 days. It would have been expected that, under these circumstances, the tolerance would have been so highly developed that a fresh heterologous superinfection would have produced no apparent change. It was found, however, that when a superinfection with strain K<sub>4</sub> was made, a distinct parasitic relapse without clinical symptoms occurred in every instance. The latter strain was isolated from a different group of monkeys from any of the former ones and apparently contained some factor absent from the other four. This strain also appeared to be the most virulent of the strains of *P. knowlesi* as yet studied in detail by us. This fact may help to account for the results obtained in superinfections with this strain.

In experiments (viii) and (ix) the animals after several previous superinfections were again superinfected with a fresh strain (K<sub>5</sub>). This produced but a mild effect.

All the above experiments were carried out with young specimens of *S. rhesus*, an animal which is highly susceptible to infections with *P. knowlesi*. Experiment (x) was made with *S. sinicus*, a species of monkey which appears to be considerably less susceptible to this infection. In the latter experiment it was observed that a superinfection with strain K<sub>4</sub>, on strains K<sub>2</sub> and K<sub>1</sub> combined, produced a milder parasitic relapse than in the case of *S. rhesus* [cf. experiments (v) to (viii)]. A later superinfection with strain K<sub>5</sub>, however, produced a more marked parasitic relapse.

As the result of the experiments analysed above, one would appear justified in concluding either that

(a) the effects of superinfection with a heterologous strain of *P. knowlesi*, combined with those of the primary infection, produced sufficient tolerance to prevent the occurrence of clinical manifestations in monkeys superinfected at a later date with *any* other heterologous strain of the same *Plasmodium*, or that

(b) some of the strains used by us in these experiments possessed some common immunological factors, the main ones of which were introduced as the result of infection with any two strains.

The latter suggestion (b) would be supported by the fact that all our original hosts (*S. irus*) were purchased, although at different times, from the same shop in Calcutta, and all were said to have been imported from Singapore. Under such circumstances it would not be surprising if similar strains, or combinations of strains, were present in these animals.

If the former suggestion (a) proved to be correct, it would have a most important bearing on the epidemiology of malaria. When, however, the animal in experiment (vii) was superinfected for the 5th time the result made this view appear much less tenable. This animal was originally infected with

strain C and had been superinfected at various times during a period of 484 days with four other strains of *P. knowlesi*. This monkey had had the opportunity of acquiring a high degree of tolerance during a primary infection lasting nearly a year. It had also had the stimulus of four later heterologous superinfections to produce an even greater tolerance. Except for one dose of stovarsol given on 403rd day for anæmia, the animal had received no specific treatment after 19th day of infection. It cannot, therefore, be suggested that the development of tolerance was inhibited by treatment. In spite of these factors, a 5th superinfection with strain K<sub>5</sub> at 523rd day resulted in a very acute infection to which the animal succumbed, although treatment with quinine and plasmoquine was given.

This finding suggests that strain K<sub>5</sub> contained a heterologous element which was absent from any of the other strains used to infect this animal. The animal behaved in the same manner as other susceptible animals do, when receiving a primary infection or a primary heterologous superinfection.

It was thought at first that a new and highly virulent species of *Plasmodium* might have been introduced, as a mixed infection, from the original host to account for this severe result. We have, however, studied this point carefully and can find no evidence to support the idea.

Another element may also have entered into this experiment. It is possible that strain K<sub>5</sub> was inoculated at the time when a relapse of the previous superinfection (K<sub>4</sub>) was about to occur. It is known that the latter strain is very liable to cause severe relapses over long periods. Under these conditions the fatal result may have been due to the combined effects of the two strains of parasite acting simultaneously. It is well recognized that a predominance of one species of *Plasmodium* may suppress the appearance of another. There is, however, no evidence that this occurs with different strains of the same parasite.

It is possible that acute infections with two different strains of *Plasmodium* at the same time may cause more severe effects than either singly. This suggestion requires further experimental work.

The results of our experiments appear to indicate that animals in nature acquire a very considerable degree of tolerance to all the local strains of *Plasmodium*, as the result of repeated infection and superinfection, if the animal survives. On the other hand, it would appear that this high degree of tolerance may not afford an effective protection against a fresh superinfection with an entirely foreign strain of the same parasite. Further experimental work will be required to confirm these findings, although they appear to be in keeping with many epidemiological observations.

These results add strong support to our previous conclusions (a) that tolerance in malaria is mainly dependent upon a specific immunity against the strain of parasite causing the infection and (b) that this tolerance is not merely a common immunity produced against all strains or species of *Plasmodium*, as the result of a general stimulation of the reticulo-endothelial system.

The findings also suggest that malaria in monkeys forms a much more suitable and comparable infection for investigating the problems of human malaria than does the malaria of birds.

#### SUMMARY OF THE RESULTS OF HETEROLOGOUS SUPERINFECTIONS.

Under the conditions of our experiments it was found that—

(1) A chronic or latent primary infection with one strain of *P. knowlesi* does not appear to confer any very effective tolerance against the clinical effects of superinfection with a heterologous strain of the same parasite. It may, however, have some ameliorating influence upon these effects.

(2) A single homologous superinfection with one strain does not appear to produce any greater tolerance to heterologous superinfection than does a simple chronic infection with the same strain.

(3) Multiple homologous superinfections may produce a slight degree of tolerance against some heterologous strains, but will not prevent the severe clinical effects produced by all such strains. It appears possible that such tolerance is only developed in the case of strains possessing some immunological factors in common.

(4) Multiple heterologous superinfections with certain strains of *P. knowlesi*, appear to produce a considerable degree of tolerance against some other heterologous strains of this parasite, but not against all strains.

(5) It appears very probable that, when multiple superinfections with several heterologous strains produce any considerable degree of tolerance to other heterologous strains, these strains have some immunological factors in common. In the absence of such common factors, multiple heterologous superinfections appear to produce no very effective tolerance.

Our thanks are due to Sub-Assistant Surgeon Harbhagwan, I.M.D., and to Laboratory Assistant Abdul Rahim for their great help in the laborious routine examinations needed in these investigations.

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## AN IMPROVISED DISSECTING MICROSCOPE FOR ENTOMOLOGICAL WORK.

BY

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[June 26, 1933.]

WHERE any considerable amount of entomological work is being carried out in connection with malariology, the need for an ample number of small dissecting microscopes has often been felt. This is especially the case where the routine dissection of a large number of anophelines is being made for the detection of malarial infections. Many organisations are unable to meet the expense involved in the purchase of the necessary instruments. To overcome this difficulty the apparatus described below was devised. Its usefulness has made it very popular with workers in Assam.

The apparatus (*vide* figure) is constructed as follows :—

(a) The base consists of a flat piece of wood, 9 inches square, covered with a layer of black paper or cloth.

(b) The supporting column for the lens is made of a wire (No. 11 S.W.G.) about 12 inches long. At one end of this a loop is formed of a size suitable for holding a Steinheil or similar lens. The wire is then bent at right angles to the loop, at a point about 7 inches from the free end.

(c) The long arm of the wire is passed through a hole bored in the base-board about 1 inch from the middle of one side. To improve the grip on the wire a strip of rubber may be placed over the hole and the wire pushed through it.

In use, the microscopic slide carrying the specimen is supported on two small mounds of plasticine. This enables the dissector to place a piece of white card below the dissection when necessary. The lens is focused by adjusting the length of wire through the hole in the board.

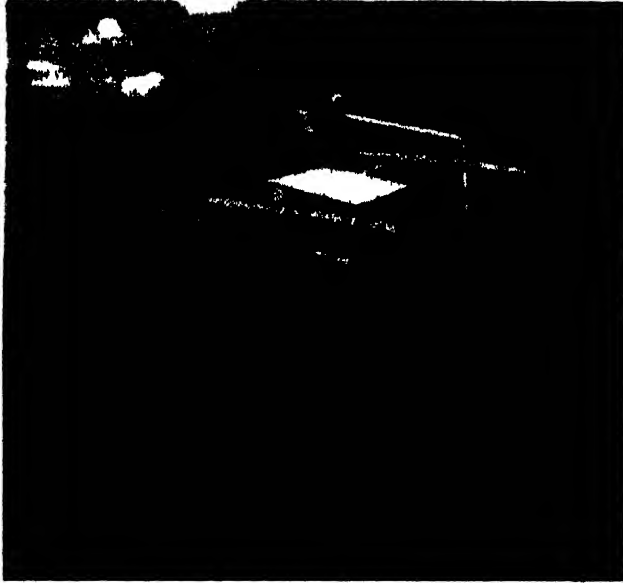


*An Improvised Dissecting Microscope.*

Possible modifications of the apparatus are :—

(a) The wire supporting the lens may be attached directly to the edge of the laboratory bench by a rubber strip.

(b) The upper portion of a microscope eyepiece ( $\times 8$  or  $\times 10$ ) may be substituted for a Steinheil lens, and has been found equally efficient.



(c) If the vertical stem of the wire be flattened, or encased in wood, it ensures greater stability of the lens.

The cost of the apparatus is little more than the initial cost of the lens used.

**ABSTRACT.**

**FURTHER INVESTIGATIONS INTO THE MALARIAL  
CONDITIONS AT KACHUGAON, GOALPARA DISTRICT,  
ASSAM, AND THE RESULTS OF ANTI-MALARIAL  
MEASURES.\***

BY

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(MS. 51 pages and one chart.)

[July 31, 1933.]

THE results of a detailed malaria survey of this hyperendemic area have been recorded in a previous paper (Gupta, *et al.*, 1932). The present article records the results of further investigations made between June 1932 and May 1933, and the effects produced by certain anti-malarial measures instituted there.

**I. FACTORS ASSOCIATED WITH THE INCIDENCE OF MALARIA.**

(a) ANOPHELINE FAUNA.

Fourteen different species of *Anopheles* were identified in the area during the period under review. A total of 9,032 adult specimens were collected. The commonest species were *A. vagus* (44 per cent), *A. minimus* (39 per cent), *A. hyrcanus* (8 per cent), *A. annularis* (*A. fuliginosus*) (4 per cent), and

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\* Copies of the original manuscript have been placed in the Office of the Director of Public Health, Shillong, Assam, and in the Library of the Malaria Survey of India, Kasauli. These are available on loan to workers who wish to consult the original.

*A. maculatus* (2·8 per cent). These species were all present throughout the year. In addition to these Anophelines less than 1 per cent of each of the following species were recorded :—*A. subpictus*, *A. aconitus*, *A. philippinensis*, *A. fluviatilis* (*A. listoni*), *A. splendidus* (*A. maculipalpis*), *A. barbirostris*, *A. kochi*, *A. culicifacies* and *A. gigas*, in order of relative frequency. *A. gigas* and *A. splendidus* were not found in the survey made in 1931.

All these species, except *A. culicifacies*, *A. splendidus* and *A. gigas*, were also collected in the larval state. A table of the relative prevalence of the different species at various seasons is given in the original. *A. minimus* and *A. vagus* were found breeding throughout the year, while in the later part of the rainy season nearly all the other species were also present.

#### (b) INFECTIVITY RATES IN ANOPHELINES.

A total of 1,530 Anophelines were dissected between June 1932 and May 1933. The numbers were as follow :—*A. minimus* 934, *A. vagus* 379, *A. annularis* 85, *A. maculatus* 74, *A. subpictus* 24 and *A. aconitus* 13. Of *A. hyrcanus*, *A. fluviatilis*, *A. splendidus* and *A. philippinensis* less than 10 specimens of each were examined. The only species found infected was *A. minimus* (5·56 per cent). In this species the oöcyst rate was 4·38 per cent and the sporozoite rate 1·28 per cent. The seasonal incidence of these infections is shown in Table I.

TABLE I.

*Results of the dissection of A. minimus.*

Month.	Number dissected.	Number with oöcysts.	Number with sporozoites.	Percentage found infected.
vi/32	93	7	4	10·70
vii/32	103	8	2	9·84
viii/32	96	6	3	8·30 *
ix/32	85	4	1	5·88
x/32	69	4	1	5·78
xi/32	197	6	0	3·04
xii/32	73	2	1	4·11
i/33	59	2	0	3·38
ii/33	45	0	0	0·0
iii/33	31	0	0	0·0
iv/33	35	0	0	0·0
v/33	48	2	0	4·16
TOTAL	934	41	12	5·56

\* One with both sporozoites and zygotes.

It was concluded from these results that *A. minimus* is the only important malaria carrier in this area.

**(c) RELATIVE INCIDENCE OF DIFFERENT SPECIES OF PLASMODIA.**

The relative incidence of the different species of malaria parasites were found to be :—(i) *P. falciparum* 60·7 per cent; (ii) *P. vivax* 13·5 per cent; (iii) *P. malariae* 0·2 per cent; (iv) mixed infections of *P. falciparum* and *P. vivax* 25·4 per cent; and (v) mixed infections of *P. vivax* and *P. malariae* 0·2 per cent.

**(d) VITAL STATISTICS.**

During 1932 a total of 228 births was recorded as compared to 178 deaths. The infantile death rate was 127·6 per mille, while the provincial figure was about 156·6. Of the total deaths 90·4 per cent was attributed to 'fever'. The 'fever death rate' was at its highest between May and October. Four cases of blackwater fever were reported with three deaths.

The figures for the total cases of 'malaria' treated in the local dispensary during the last 7 years have been tabulated. These indicate that, although 1932 was considered to be a bad malarious year, the cases were fewer than in any year except 1930. 'Malaria' cases formed 19·9 per cent of all patients treated, as compared with an average of 27·68 for the province as a whole.

**(e) METEOROLOGY.**

The rainfall for 1932 was 176·87 inches, which is above the average. June was the wettest month with 61·87 inches of rain. The original paper gives a graph showing the relation of malaria cases, rainfall and infectivity rate in Anophelines.

**II. EFFECTS OF CURATIVE TREATMENT.**

An extensive investigation was carried out into the effects of both curative and prophylactic treatment with quinine, plasmoquine and atebrian under field conditions.

**(a) EFFECTS OF TREATMENT WITH QUININE AND PLASMOQUINE.**

The patients were divided into three classes :—(1) acute cases, (2) relapsing cases, and (3) chronic relapsing cases. The routine treatment administered was as follows :—

Preliminary purgation with calomel and salines was given in all cases. The specific treatments were :—

(1) *In acute cases.*—Quinine, 12 grains, and plasmoquine, 1/6th grain, were given twice daily after food for 7 days.

(2) *In relapsing cases.*—(i) If these were due to *P. falciparum*, the patients were treated like the acute cases for 1 week, but, although the same dosage of quinine was continued for another week, plasmoquine was omitted in the latter period. (ii) If due to *P. vivax*, or to a mixed infection, the treatment used for acute cases was continued for 2 weeks.

(3) *In chronic relapsing cases.*—These received the same treatment as the acute cases during the first week, but during the second and third weeks, the quinine was reduced to 16 grains daily and the plasmoquine continued at 1/6th grain. All anæmic patients were given tonic treatment.

The bloods of all patients were examined before treatment started. The acute cases were re-examined on 4th and 8th days; the relapsing cases on 15th day also, and the chronic ones again at the end of third week.

The results of these courses of treatment are summarized in Table II.

TABLE II.

*Results of curative treatment with quinine and plasmoquine.*

Type of infection.	Number of cases.	DIAGNOSIS OF INFECTION.						Cured.	Died.	Number stopping treatment.	Number of relapses.	Percentage of relapses.
		M.T.*	B.T.*	Q.t.*	M.T. and B.T.*	B.T. and Q.t.*	Clinical only					
(1) Acute	370	183	47	1	71	1	67	346	2	22	37	10
(2) Relapsing	37	20	3	..	12	..	2	37	..	..	..	..
(3) Chronic	37	19	4	..	9	..	..	33	..	4	..	..

\* M.T. = *P. falciparum*. B.T. = *P. vivax*. Q.T. = *P. malariae*.

Blood examinations made on 4th day in the 370 acute patients showed *P. falciparum* in 52 cases, *P. vivax* in 15 and mixed infections of these two species in 20. The positive findings on 8th day were 4, 0 and 1 respectively.

The 37 relapsing cases occurred after the termination of the treatment of acute cases. Some of the relapses occurred as late as 90th day, and it was therefore difficult to exclude the possibility of a reinfection during this period. Blood examinations made on 4th day of the second course of treatment, showed *P. falciparum* in 4 cases and a mixed infection of this parasite with *P. vivax* in 1. No parasites were found at the end of treatment.

All the chronic relapsing cases suffered from distinct anæmia and from splenic enlargement. Of these 10 still showed *P. falciparum* on 4th day of treatment, 2 showed *P. vivax* and 1 a mixed infection with these two parasites. No parasites could be detected at the end of treatment.

Extensive tables are given in the original of the results of parasite counts (numbers of parasites per 1,000 leucocytes). These were made during the course of treatment in a large number of the acute and of the relapsing infections.

It was concluded as the result of these experiments that (i) the duration of treatment in malaria could be conveniently shortened by adding plasmoquine to quinine, and (ii) that this treatment considerably reduced the relapse rate.

(b) EFFECTS OF TREATMENT WITH ATEBRIN AND PLASMOQUINE.

The course of treatment used for adults was one tablet of atebirin (1½ grains) and one tablet of plasmoquine (1/8th grain) thrice daily after food for 4 days. The patient was prepared for treatment by the usual course of calomel and magnesium-sulphate purgation.

Sixty-one cases were treated. Of these 36 were infections with *P. falciparum*, 2 with *P. vivax*, 17 with both these parasites and 6 were diagnosed as malaria from clinical manifestations only. No death occurred and 60 were discharged as cured. One case showed slight intolerance-nausea, yellow coloration of the body, etc., but these effects quickly disappeared after the cessation of treatment. Eight cases relapsed at a later date. Of these 4 were due to *P. falciparum*, 2 to mixed infections of this parasite and *P. vivax*, and in 2 the diagnosis of relapse was a clinical one.

At the end of treatment 6 acute cases and 2 relapsing ones still showed parasites in the peripheral blood. In 6 cases the parasites were *P. falciparum* and in the other 2 mixed infections.

As the result of these investigations it was concluded that—

- (i) In benign tertian malaria a 4-days' course is sufficient.
- (ii) The relapse rate is slightly higher than with quinine and plasmoquine combined, but a longer course of treatment might reduce this rate still more.
- (iii) Atebrin can be substituted for quinine with advantage.
- (iv) The duration of treatment can be shortened by the use of atebirin combined with plasmoquine.
- (v) Atebrin can be used for mass treatment in the field under medical supervision.

### III. THE EFFECTS OF PROPHYLACTIC TREATMENT. -

(a) PROPHYLACTIC TREATMENT OF ADULTS WITH QUININE AND PLASMOQUINE

The prophylactic treatment of adult Government servants was commenced in June 1932 and still continues.

*Treatment.*—During June, July and August 1932, plasmoquine alone was used, in doses of one-third grain twice weekly. As the results obtained were not so satisfactory as expected, this dose of plasmoquine was reinforced with 4 grains of quinine on each occasion from September 1932 onwards.

The effects of this treatment on the incidence of fever have been tabulated and compared with the incidence in an untreated control population. These results are summarized in Table III.

As a result of these experiments it was concluded that the number of febrile attacks of malaria in an adult population can be reduced by regular, bi-weekly, prophylactic courses of quinine and plasmoquine, even in a hyper-endemic area. The opinion formed was that the fever incidence would be still further reduced by daily administration of these drugs.

TABLE III.

*Results of prophylactic treatment with quinine and plasmoquine.*  
(Adults.)

Months and years.	TREATED POPULATION.				CONTROL POPULATION.			
	Average number of persons per month.	Treatment.	Total cases of fever.	Percentage per mensem.	Average number of persons per month.	Treatment.	Total cases of fever.	Percentage per mensem.
vi/32 to viii/32 ..	122	P.*	40	10.9	85	nil	45	26.4 ‡
ix/32 to iv/33 ..	112	PQ. †	41	4.6	85	nil	71	10.4

\* P. = Plasmoquine. † PQ. = Plasmoquine and Quinine. ‡ June and July 1932 only.

(b) PROPHYLACTIC TREATMENT OF CHILDREN WITH QUININE AND PLASMOQUINE.

Before the prophylactic course was started all the children were treated twice daily for one week, in an attempt to free the peripheral blood of asexual parasites. The same mixture and proportionate doses were given as those described below.

*Treatment.*—The stock mixture used was:—Euquinine 10 grains, plasmoquine 1/6th grain, milk 10 drms. The dosage prescribed according to age was:—Below 1 year, 1 drm.; 1 year, 2 drms.; 2–4 years, 3 drms.; 5–10 years, 4 drms.; 10–12 years, 5 drms.

During the period of prophylactic treatment appropriate doses were given twice weekly.

The spleen rate was observed in the children before treatment commenced, and at 4-monthly intervals thereafter. Absence of sufficient staff prevented routine blood examinations being made.

In September 1932, among 210 children the spleen rate was 89.5 per cent before treatment; in January 1933, it was 56.2 per cent among 199 children, and in May 1933, it was 47.4 per cent among 179 children. In a control village, where no treatment was used, the rate remained constant about 88.2 per cent. Apart from the reduction in spleen rate, a marked reduction in the size of the spleen was noted in about 95 per cent of children.

As a result of these experiments it was concluded that it was practicable, in the case of a controlled population with active co-operation, to free an area of gametocyte carriers and to reduce the spleen rate very considerably.

Continual changes in the population, however, militate against the chance of obtaining these results.

#### **IV. ANTI-MOSQUITO MEASURES.**

Antilarval measures were carried out within a radius of half a mile around Kachugaon. The work started in July 1932, and is still in progress.

Oiling was used on all permanent breeding places once weekly. Temporary breeding places, rice-fields and the edges of streams were treated with paris green. These measures were found to be very expensive, chiefly because of the high cost of transportation of materials.

Clearance of scrub and vegetation in the vicinity of dwellings was carried out, to reduce the shelter for adult mosquitoes.

The results of the anti-mosquito campaign were evaluated by means of adult catches made weekly in 4 stations, situated at different corners of the area. The results have been tabulated in the original paper and it is recorded that the total catch fell from 276 per week in June 1932 to 20 at the end of April 1933.

#### **V. COST OF THE CAMPAIGN.**

The cost of the campaign was Rs. 7,500 for the year. It is suggested that the work could be continued at a much cheaper rate if the Sub-Assistant Surgeon in charge of the local dispensary received training in the work. He would then be able to superintend the campaign in addition to his other duties, and thus the cost of personnel would be considerably reduced.

#### **VI. RECOMMENDATIONS.**

The following are the chief recommendations made :—

(1) A continuation of the present system of curative and prophylactic treatment—(i) curative treatment for all cases; (ii) prophylactic treatment for children throughout the year; and (iii) prophylactic treatment for adults from April to October.

(2) Continuation of anti-mosquito measures throughout the year.

(3) The campaign be placed in charge of the Sub-Assistant Surgeon of the local dispensary, under the supervision of the Public Health Department.

(4) A slight increase in the subordinate staff.

(5) Vigorous propaganda to rouse the public health conscience and gain the co-operation of the local people more effectively.

#### **REFERENCE.**

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